E. R. Garrison

JOURNAL OF DAIRY SCIENCE

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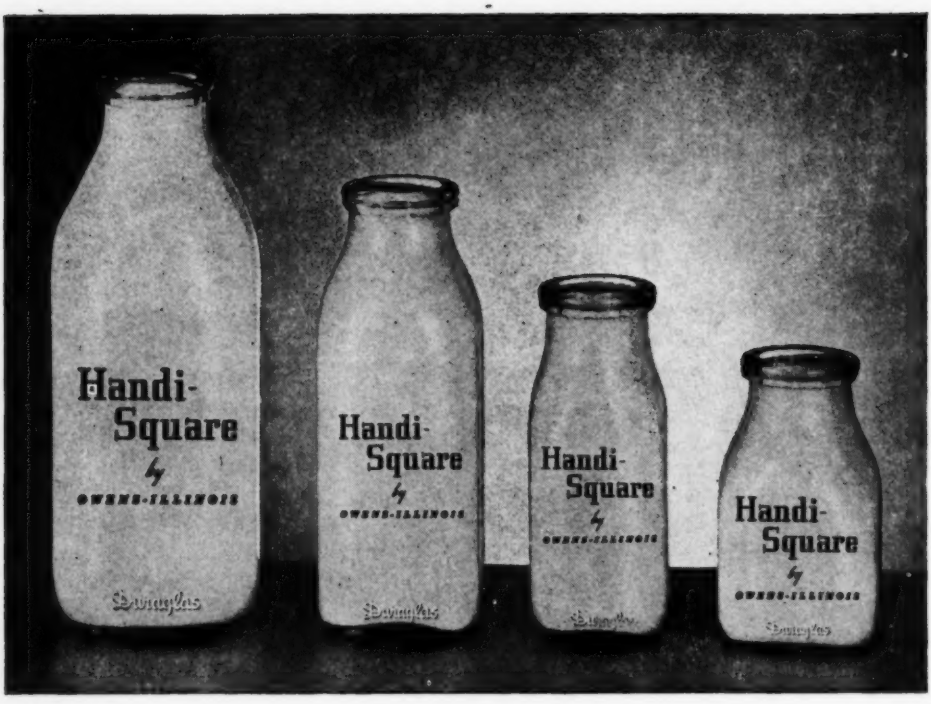

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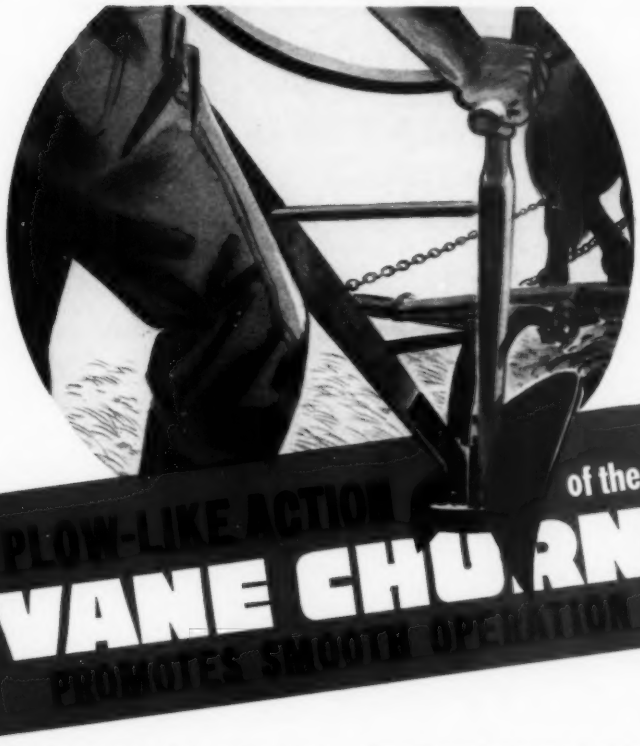

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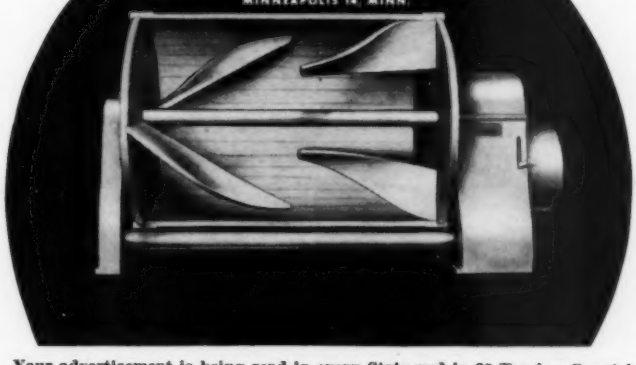

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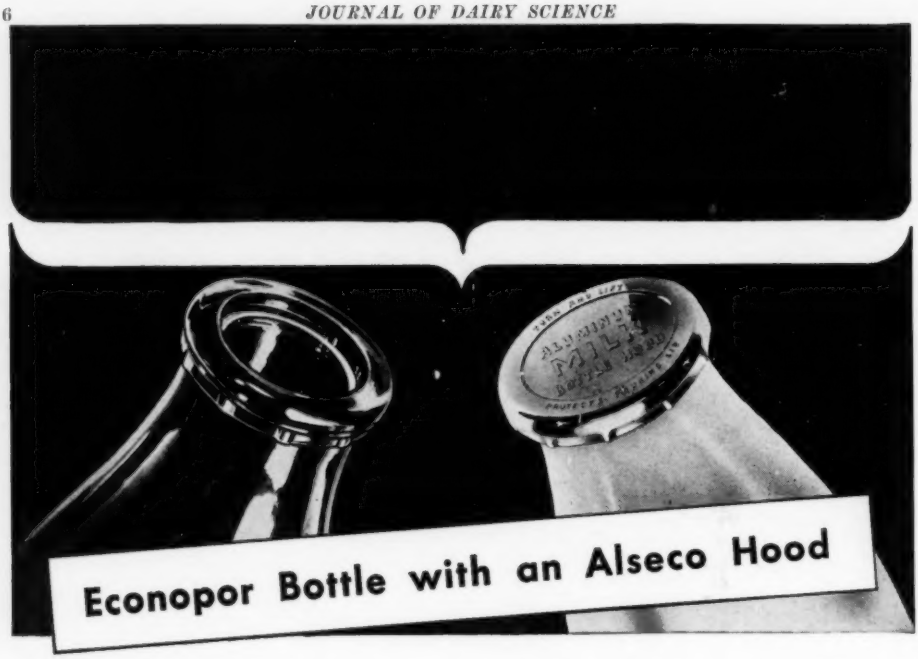

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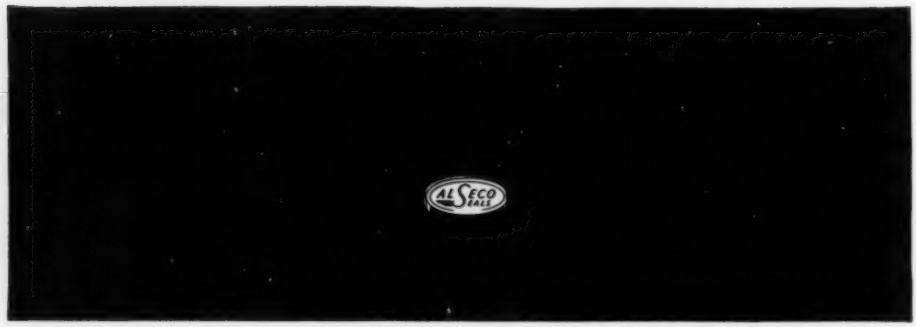

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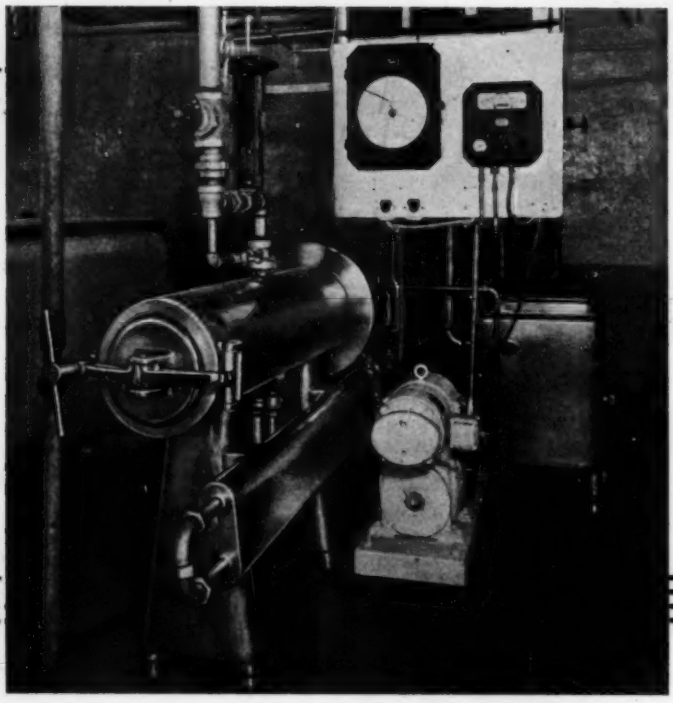

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THE PREPARATION AND EFFECT OF HEAT TREATMENT ON THE WHEY PROTEINS OF MILK[1]

H. A. HARLAND[2] AND U. S. ASHWORTH

Division of Dairy Husbandry, Agricultural Experiment Station, Pullman, Washington

Many methods used for the preparation of heat-labile proteins of milk involve the use of one or more of the common inorganic salts. Palmer (4) employed sodium sulfate for the preparation of a crystalline lactoglobulin fraction of the whey proteins of milk. The Sorensens (7) have used ammonium sulfate in very extensive fractionation studies on whey proteins. Magnesium sulfate and sodium chloride (2) were used for the first isolation of lactoglobulin from milk. Although it is common knowledge that casein is precipitated from milk saturated with sodium chloride, the authors know of no instance in which sodium chloride and acid have been used for the preparation or quantitative estimation of whey proteins.

Numerous papers have been published concerning the effect of heat treatment on the nitrogen distribution in milk. In general, the results agree only in a qualitative way because of variations in treatment, methods of analysis, and milk samples used. One of the most critical studies of the effect of heat treatment on heat-labile proteins of milk was made by Rowland (5). He determined the amounts of lactoglobulin and lactalbumin denatured on heating milk at temperatures of from 63° to 80° C. for periods of from 2.5 to 60 minutes. Menefee, Overman and Tracy (3) have published a rather extensive article on the effect of processing on the nitrogen distribution of milk. Ashworth and Van Orden (1) found that whey proteins of non-fat dry-milk solids are largely denatured and are precipitated with casein in the analysis of the milk.

The heat-labile proteins of milk have long been an object of study by those interested in the production of non-fat dry-milk solids for use by the baking industry. It was in the course of such a study that the data of this paper were obtained. The purpose of this work was to find a method for

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the preparation of relatively undenatured whey proteins that would be free of ions likely to affect experimental baking results. This method was used to study quantitatively the effect of heat treatment on the whey proteins of separated milk. These quantitative data are compared with that obtained by Rowland's (6) method.

EXPERIMENTAL MATERIALS AND METHODS

The milk used in this investigation was taken from a bulked supply of Grade A milk. The samples were refrigerated at 0°–5° C. when it was not practical to complete the processing and analysis within a few hours. All of the data represent the average of closely agreeing complete sets of treatments and analyses from two or more lots of the bulked milk. Since rather large amounts of sodium chloride were utilized in the course of this and related work, the use of a good grade of cheese salt was investigated. The cheese salt proved to give identical results with U.S.P. quality sodium chloride; so the less expensive salt was used for all subsequent work.

The whey proteins were prepared by saturating three gallons of separated milk with 3700 grams of sodium chloride at 40° C. After being allowed to stand overnight, approximately 100 grams of filter-aid[3] were stirred into the mixture. After standing about one hour the casein was filtered off with suction through a large Buchner funnel fitted with a Whatman No. 1 filter paper covered to a depth of 1/8 of an inch with filter-aid. The whey proteins were precipitated from the filtrate by the addition of 10 per cent hydrochloric acid with rapid stirring to pH 2.0. Following standing overnight at room temperature, the whey protein was filtered through an E and D no. 193 folded filter paper. The whey proteins were removed from the filter paper and transferred with a minimum of water to a cloth reenforced cellophane dialyzing sack and dialyzed against distilled water for 72 hours at 0°–5° C.

The heat treatments were done in a thermostatically controlled bath of rapidly circulating water. The use of 180 × 20 mm. thin-walled test tubes permitted rapid heating and cooling of the samples. These tubes were especially satisfactory for this purpose since a relatively small surface of the milk was exposed and successive samples were readily obtained by removing one or more tubes from the bath.

Rowland's (6) methods of analysis were followed where indicated except that larger quantities of samples and reagents were employed to permit the use of macro-Kjeldahl procedures; also the milk samples taken for analysis were accurately measured at 20° C. by means of pipettes rather than by weighing.

For the determination of the volume of the salt in a saturated solution of milk and sodium chloride, 26 grams of the latter were added to a 100 ml.

[3] The filter-aid used was "Hyflo Super-cel," a Johns-Manville product.

volumetric flask containing 75 ml. of milk. After warming to 40° C. with occasional shaking, the mixture was allowed to stand overnight at room temperature. Entrapped air was removed by means of a water aspirator pump and the volume made up to 100 ml. with 15.5 ml. of a saturated solution of sodium chloride. Thus the change in volume due to sodium chloride was 9.50 ml. per 75 ml. of the saturated milk. Each ml. of the filtrate resulting from the saturation of separated milk with sodium chloride was equivalent to 0.887 ml. of the original milk. Saturation of 50 and 85 ml. aliquots of milk with sodium chloride and making up to 100 ml. with a saturated solution of the salt also resulted in the conversion factor of 0.887. Rowland's (6) correction factor of 0.995 for the volume of the casein and fat was not applied since separated milk was used in this work and the small correction for the volume of the casein would have no material effect on the results.

To determine the non-casein N in separated milk by saturation with sodium chloride, 26 g. sodium chloride were shaken with 75 ml. of milk in a stoppered 125-ml. Erlenmeyer flask. The mixture was heated to 40° C. in a water bath to assure rapid and complete saturation of the milk and allowed to stand overnight at room temperature. Approximately 1 g. of filter-aid was shaken with the mixture a few minutes before filtering with suction through a Whatman No. 1 filter paper covered to a depth of 1/8 of an inch with filter-aid in a 9-cm. Buchner funnel. Two 20-ml. aliquots of the filtrate were taken for the Kjeldahl N determination.

The non-protein N was determined by the acidification of the filtrate containing the non-casein N. Since it was often desirable to determine the non-casein N and non-protein N on the same aliquot, 200 ml. of the milk were saturated with 70 g. of sodium chloride and the filtrate used for both determinations. For the non-protein N, 100 ml. of the filtrate were treated with 1.50 ml. of 10 per cent HCl and allowed to stand overnight. After the addition of 1 g. of filter-aid, the whey proteins were filtered off on a folded Whatman No. 1 paper and 25-ml. aliquots of the filtrate were taken for the Kjeldahl N determination.

EXPERIMENTAL RESULTS

Preparation and purification of whey proteins. Whey proteins were prepared as described under Materials and Methods. The dialyzed material contained between 8 and 10 mg. of protein N per ml. Dialyzing for 72 hours reduced the sodium chloride content to less than 3 per cent of the weight of the protein. The pH of the dialyzed proteins remained somewhat below that of normal milk, but was readily adjusted with alkali. The clear, slightly colored protein solutions were preserved for further experimentation by saturating with toluene and storing at 0–5° C. The yield of whey proteins by this procedure is approximately 95 per cent of the total present in the separated milk.

Effect of pH on the sodium chloride milk filtrate. The precipitation of whey proteins with hydrochloric acid from the casein-free filtrate resulting from the saturation of separated milk with sodium chloride is indicated by the pH versus mg. of N precipitated in figure 1. The initial pH of the filtrate was invariable near 6.2. The addition of 10 per cent HCl causes the precipitation of the whey proteins to begin at pH 5.0. The precipitation of the whey proteins is practically complete at about pH 3.8, reaching a maximum at approximately pH 2.0. At the latter pH there remained 0.28 mg. of non-casein N per ml. of milk. This value is slightly lower than any of the similar values obtained by Rowland's method for non-protein N as shown by the data in table 1.

Comparison of the two methods of analysis of separated milk. Data resulting from determinations of non-casein and non-protein N in six separate lots of pasteurized separated milk by both Rowland's method and the sodium chloride-hydrochloric acid methods are shown in table 1. The average per cent of the total N appearing as non-casein N is 21.7 for Rowland's method compared to 18.8 for sodium chloride. Rowland's method for non-protein N accounts for 5.64 per cent of the total N and the sodium chloride-hydrochloric acid method, 5.49 per cent.

TABLE 1

Comparison of Rowland's and sodium chloride–hydrochloric acid methods of analysis of pasteurized separated milk

Lot No.	Total N (mg. per ml.)	Non-casein N (mg. per ml.)		Non-protein N (mg. per ml.)	
		Rowland's	NaCl–HCl	Rowland's	NaCl–HCl
1	5.93	1.24	1.06	0.31	0.29
2	5.46	1.24	1.02	0.30	0.30
3	5.50	1.21	1.00	0.30	0.28
4	5.67	1.18	1.08	0.32	0.32
5	4.57	1.15	0.99	0.29	0.29
6	6.14	1.27	1.07	0.29	0.30
Average per cent of total N		21.73	18.79	5.64	5.49

The use of sodium chloride and hydrochloric acid for the estimation of heat-denatured proteins. The sodium chloride and Rowland's methods of analysis were used to determine the effect of heat treatment on whey proteins as indicated in table 2. The heat treatments to which the milk was exposed are given in the first column of the table. The values for whey proteins in column 2 represent the difference between the non-casein N and the non-protein fractions. The weights of whey coagulated[4] or denatured by the heat treatments given in the third column are calculated from the data in column

[4] The terms coagulated and denatured have been used interchangeably throughout this paper. Often the term denatured refers to proteins that have undergone changes that precede actual coagulation.

2 by subtracting the weight of whey proteins after heat treatment from the corresponding values for the milk having no heat treatment. Likewise the per cent of the whey proteins affected by heat is calculated from the data in column 2.

The effect of heat treatment on whey proteins. The effect of time and temperature of heat treatment on the amount of whey proteins denatured according to the sodium chloride method of analysis is graphically illustrated

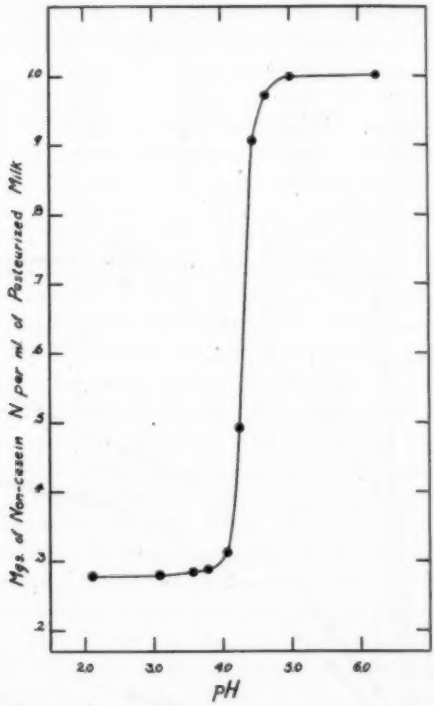

FIG. 1. The precipitation of whey proteins with hydrochloric acid from the casein-free filtrate resulting from the saturation of separated milk with sodium chloride.

in figure 2. The data were obtained on pasteurized milk and converted to raw milk basis by making use of data for raw and pasteurized milk shown in table 2. This conversion is based on the assumption that all the whey protein normally coagulated by pasteurization is coagulated during these special treatments. This may not be strictly correct for the five-minute, 70° and 75° C. treatments but the error would not alter the curves appreciably. The 85° and 95° C. treatments, especially for periods of over five minutes, may introduce other factors such as hydrolysis of the casein and whey proteins which tend to distort the denaturing effect of heat on the whey proteins.

TABLE 2

Comparison of sodium chloride–hydrochloric acid and Rowland's methods for the determination of heat-denatured whey proteins

Heat treatment		Whey proteins (mg. per ml.)		Weight of whey proteins coagulated (mg. per ml.)		Per cent of whey proteins coagulated	
Temp. (° C.)	Time (min.)	Rowland's	NaCl–HCl	Rowland's	NaCl–HCl	Rowland's	NaCl–HCl
None		0.978	0.782	.....	.....	.....	.....
Holder pasteurization		0.918	0.737	0.060	0.045	6.13	5.76
75	20	0.511	0.338	0.467	0.444	47.7	56.8
75	45	0.368	0.184	0.610	0.598	62.3	76.4
80	45	0.238	0.051	0.740	0.731	75.7	93.5
85	30	0.217	0.035	0.761	0.747	77.8	95.6
95	10	0.222	0.036	0.756	0.746	77.2	95.3

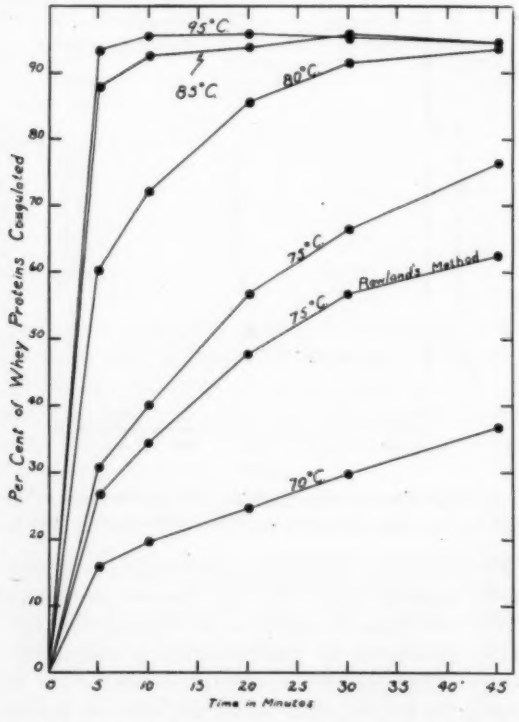

FIG. 2. The effect of heat treatment on the per cent of coagulation of the whey proteins of separated milk.

DISCUSSION

The treatment of separated milk with sodium chloride and hydrochloric acid readily yields soluble whey protein free of all ions not normally present in milk. These two characteristics of the whey proteins prepared by this method are particularly important when the material is to be used in experimental baking. Trace quantities of most of the substances normally used in the isolation of proteins often greatly affect experiments involving microorganisms. Although dry protein material was not desired in our work, the concentrated protein solution (approximately 10 times the whey protein concentration of separated milk) would be a satisfactory starting point for any of the several drying methods.

The data of table 1 show that the sodium chloride method of analysis gives lower non-casein N values and consequently higher casein N values than does Rowland's method. The differences in the amount of non-casein N are due entirely to the whey proteins since there is no significant differences between the non-protein N by the two methods. The average value of 21.73 per cent for the non-casein N as determined by Rowland's method is near the values reported in the literature (3, 6). The sodium chloride method results in 17.3 per cent less whey protein N than obtained by Rowland's method.

The data in table 2 illustrate the fact that although the two methods of analysis do not give the same results for the per cent of whey proteins denatured by heat, the weight of the protein denatured is nearly the same in both cases. The small differences are well within the experimental error. The amount of whey proteins coagulated reach a near maximum following treatment at 80° C. for 45 minutes. Shorter periods of treatment at higher temperatures yield similar results. These results clearly indicate that the use of sodium chloride as the precipitating agent for casein results in practically complete removal of all proteins from milk not readily subject to heat denaturation. This might suggest the possibility of globulin being precipitated with casein were it not for the fact that Sebelin and Emmerling, according to Davies (2), isolated globulin from the filtrate of milk saturated with sodium chloride.

It appears that all of the proteins contained in the filtrate resulting from the saturation of separated milk with sodium chloride are precipitated within a small range of pH as demonstrated by figure 1. Preliminary attempts to fractionally precipitate the proteins within this small range have not been entirely successful.

The heat treatment studies illustrated in figure 2 show that when it is desirable to denature a large per cent of the whey proteins heat treatment at 80° C. for 45 minutes or a higher temperature for shorter periods is necessary. Those requirements for heat denaturation of whey proteins are approximately the same as those used in commercial heat treatment of separated milk for drying purposes.

SUMMARY

The preparation of a concentrated solution of whey proteins from separated milk by treatment with sodium chloride and hydrochloric acid is described. The method yields relatively undenatured and pure protein free of all substances foreign to milk.

A method is described for the quantitative estimation of the heat labile proteins of milk involving saturation of the milk with sodium chloride and acidification of the filtrate with hydrochloric acid to pH 2.0. This method yields lower whey protein and higher casein values than the generally accepted method of Rowland (6).

The effect of time and temperature of heat treatment on the whey proteins has been studied. Treatment at 80° C. for 45 minutes or higher temperatures for shorter periods denature 93–95 per cent of the whey proteins as determined by the sodium chloride–hydrochloric acid method.

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ACTION OF PENICILLIN AGAINST MASTITIS ORGANISMS IN MILK

H. W. SEELEY, JR., E. O. ANDERSON AND W. N. PLASTRIDGE*
Storrs Agricultural Experiment Station, University of Connecticut, Storrs, Conn.

The successful application of penicillin to diverse human infections insures its widespread use in attempts to combat bovine mastitis. Two reports (1, 5) contraindicate the use of penicillin intravenously in the treatment of mastitis. Using the technique of udder infusion Bryan *et al.* (1) have reported a good measure of success in the treatment of quarters infected with *Streptococcus agalactiae*. Somewhat less promising results have been noted by Kakavas (3), and on staphylococcal infections by Klein and his associates (4).

Similarity of the composition of blood and milk would seem to forecast a favorable antibacterial action of penicillin in a milk menstruum. The present experiment: 1. Tested the effectiveness of penicillin against organisms commonly associated with udder infections, and 2. Compared the action of penicillin in beef infusion broth, in which it is known to be effective, to its action in milk.

EXPERIMENTAL

The test organisms were recent isolations from cases of udder infections, with the exception of *Corynebacterium pyogenes* (Atwater), *Staphylococcus aureus* (298) and a hemolytic group C streptococcus ("animal pyogenes"). These three are stock cultures originally isolated from mastitic milk. Prior to exposure to penicillin, the organisms were carried in litmus milk transfers for several days. The final transfers which served as inocula were actively growing 18-hour cultures.

A sodium salt of penicillin[1] (assayed at 909 Oxford units per mg.) was weighed aseptically and dissolved in skimmed milk which had been previously sterilized by intermittent heating. A fresh solution, prepared just before each experiment, served as a source of supply from which the proper amounts of penicillin could be conveniently removed.

To appropriate amounts of sterile skimmed milk adjusted to pH 7.0 inocula of the test organisms and penicillin were added in predetermined quantities to give a total of five ml. of penicillin-culture mixture. A relatively wide range of concentrations of the drug was first selected. The range was narrowed in later experiments and only the critical portions of the data are included in this report. The penicillin-culture mixtures were held at 37° C. At 24-hour intervals they were removed from the incubator and

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[1] Supplied by courtesy of E. R. Squibb and Sons.

streaked on five per cent ox-blood-agar plates which in turn were incubated at 37° C. for 24 hours and examined for colonial growth. Plates streaked with the slow-growing *C. pyogenes* were examined after an additional 24-hour incubation period.

The results recorded in the accompanying tables show the sterilizing action of penicillin in terms of the growth of the exposed organism on blood-agar. Observations on each culture were extended for seven days although after four days exposure no additional changes occurred.

TABLE 1

Action of graded concentrations of penicillin on large inocula of mastitis organisms in milk

Units penicillin per ml. medium	*Str. agalactiae* 1,250,000 per ml.				*Str. dysgalactiae* 200,000 per ml.				*Str. uberis* 200,000 per ml.			
	Days after inoculation				Days after inoculation				Days after inoculation			
	1	2	3	4	1	2	3	4	1	2	3	4
99	∓	0	0	0	0	0	0	0	0	0	0	0
49	+	∓	0	0	0	0	0	0	0	0	0	0
9	+	+	∓	0	0	0	0	0	+	∓	0	0
	S. aureus 30,400,000 per ml.				*C. pyogenes* 30,000,000 per ml.				*E. coli* 6,500,000 per ml.			
	Days after inoculation				Days after inoculation				Days after inoculation			
	1	2	3	4	1	2	3	4	1	2	3	4
99	0	0	0	0	0	0	0	0	+	+	+	+
49	+	∓	0	0	+	∓	0	0	+	+	+	+
9	+	+	∓	0	+	+	0	0	+	+	+	+

+ = Good growth of transfer on blood-agar.
∓ = Few colonies.
0 = No growth.

In the first experiment (table 1) large inocula of both the test organisms and penicillin were employed. In subsequent experiments the inocula of the test organisms were maintained between 25,000 and 40,000 organisms per ml. and the concentrations of penicillin were graded downwards from a maximum of 10 units.

Table 2 shows the comparative action of penicillin in broth and in milk inoculated in parallel, and table 3 shows the numbers of units required to prevent transfers of the strains tested from growing on blood-agar. The results of table 3 were duplicated in another trial.

Throughout the experiment the pH levels of the media were adjusted to 7.0 to simulate the pH of milk from infected quarters.

DISCUSSION OF RESULTS

All organisms tested were susceptible to small amounts of penicillin

TABLE 2

Comparative action of penicillin on mastitis organisms in milk and in broth at pH 7.0

Units penicillin per ml. medium	*Str. agalactiae* 30,000 per ml.								*Str. dysgalactiae* 35,000 per ml.								*Str. uberis* 35,000 per ml.							
	Days after inoculation								Days after inoculation								Days after inoculation							
	1		2		3		4		1		2		3		4		1		2		3		4	
	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B
0.18 to 10.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.09	0	0	∓	0	+	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.03	+	0	+	0	+	0	+	+	0	0	0	0	0	0	0	0	+	∓	+	+	+	+	+	+
0.016	+	+	+	+	+	+	+	+	0	+	∓	+	+	+	+	+	+	+	+	+	+	+	+	+
	S. aureus 30,000 per ml.								*C. pyogenes* 25,000 per ml.								*E. coli* 30,000 per ml.							
	Days after inoculation								Days after inoculation								Days after inoculation							
	1		2		3		4		1		2		3		4		1		2		3		4	
	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B
0.18 to 10.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+	+
0.09	0	0	0	+	+	+	+	+	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+	+
0.03	∓	∓	+	+	+	+	+	+	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+	+
0.016	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+	+

M = Milk; B = Broth; 0 = No growth.
∓ = Few colonies; + = Good growth of transfer on blood agar.

except *Escherichia coli.* This particular culture was obtained from an acute case of mastitis and 99 units failed to check its growth in milk.

Variation in the resistance to penicillin of strains of the same species was slight except in the case of *Streptococcus uberis* where one strain required 0.03–0.09 units per ml. of medium to render the organisms nonviable, and the other required 0.18–0.36 units. Both the stock strain and the freshly isolated strain of *C. pyogenes* required 0.008–0.016 units to render the culture nonviable. It was noted that a borderline difference existed between the susceptibility of the stock strain and the freshly isolated strain of *S. aureus,* the latter being slightly more resistant.

Str. uberis and *Str. agalactiae* were somewhat more resistant than *Streptococcus dysgalactiae* and the hemolytic group C streptococcus. Where the

TABLE 3

*Action of penicillin on mastitis organisms in milk**

Organism	Oxford units preventing growth after exposure for			
	1 day	2 days	3 days	4 days
Str. agalactiae (A)	0.03 –0.09	0.09 –0.18	0.09 –0.18	0.09 –0.18
" " (B)	0.03 –0.09	0.03 –0.09	0.03 –0.09	0.03 –0.09
Str. dysgalactiae (A)	0.008–0.016	0.016–0.03	0.016–0.03	0.016–0.03
" " (B)	0.016–0.03	0.016–0.03	0.016–0.03	0.016–0.03
Str. uberis (A)	0.03 –0.09	0.03 –0.09	0.03 –0.09	0.03 –0.09
" " (B)	0.18 –0.36	0.18 –0.36	0.18 –0.36	0.18 –0.36
Hemolytic group C Streptococcus	0.016–0.03	< 0.016	< 0.016	< 0.016
Str. viridans	0.09 –0.18	0.03 –0.09	0.03 –0.09	0.03 –0.09
S. aureus (A)	0.03 –0.09	0.03 –0.09	0.09 –0.18	0.09 –0.18
" " (#298)	0.09 –0.18	0.03 –0.09	0.03 –0.09	0.03 –0.09
C. pyogenes (Atwater)	0.008–0.016	0.008–0.016	0.008–0.016	0.008–0.016
" " " (A)	0.008–0.016	0.008–0.016	0.008–0.016	0.008–0.016
E. coli	> 10.0	> 10.0	> 10.0	> 10.0

* Inocula were adjusted to give from 25,000–40,000 organisms per ml. milk.

serological groupings are comparable our results agree closely with those of Watson (6) who, working with organisms of the Lancefield groups, found that 0.0078 to 0.0313 units were required to prevent the growth of group C streptococci. In our experiment *Str. dysgalactiae,* which is agglutinated by group C antiserum, was rendered nonviable by 0.016–0.03 units. Similarly, *Str. agalactiae* (group B) was rendered nonviable by Watson with 0.0625 units and in the present experiment one strain did not initiate growth after exposure to 0.03–0.09 units and the other after exposure to 0.09–0.18 units.

The *Streptococcus viridans* culture was obtained from a single quarter showing a temporarily increased leucocyte count. A concentration of 0.03–0.09 units of penicillin prevented its growth in milk after two days' exposure.

Foley, Lee and Epstein (2), using diluted, partially purified preparations and crude liquors containing penicillin, reported that in brain-heart

medium *S. aureus* was prevented from growing by 0.02–0.15 Florey units, *Str. agalactiae* by 0.02–0.17 units and *Str. dysgalactiae* by 0.02 units. These investigators present data to show that a population of 2.3 millions of *Str. agalactiae* in milk was destroyed in about 3.5 hours by one Florey unit of penicillin, and point out that fresh milk, which contains lactenin, is a more favorable medium for the action of penicillin than boiled milk in which the lactenin has been destroyed.

It is difficult to translate *in vitro* results, which probably represent optimum conditions for the action of the drug, into terms of a therapeutic dose. In a milk medium, however, with the exception of *E. coli* (and probably other species of this group), the common agents of infectious mastitis are rendered nonviable by extremely small amounts of penicillin.

SUMMARY

1. A comparative *in vitro* study of the action of penicillin against the common mastitis organisms suspended in sterile milk and broth indicated that milk, at pH 7.0, is as favorable a medium for penicillin action as buffered beef infusion broth.

2. Arranged as follows in order of decreasing resistance to the action of penicillin, *Str. agalactiae* and *Str. uberis*, *S. aureus* and *Str. viridans*, *Str. dysgalactiae* and *C. pyogenes* and the hemolytic group C streptococcus were successfully rendered nonviable *in vitro* in milk with small amounts of penicillin.

3. A strain of *E. coli* isolated from an acute case of mastitis was refractory to penicillin in concentrations up to 99 units.

4. Strains within a species were susceptible in most instances to approximately the same concentrations of penicillin.

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PREDICTING THE TRANSMITTING ABILITY OF YOUNG DAIRY SIRES FOR MILK PRODUCTION, BUTTERFAT TEST, AND BUTTERFAT PRODUCTION[1]

H. C. DICKEY AND PEDRO LABARTHE[2]
Colorado Agricultural Experiment Station, Fort Collins, Colorado

The selection of young bulls as future dairy herd sires has long been a problem of dairy cattle breeders. About the only criteria which can be used in selecting young bulls are (a) pedigree and (b) individuality. With older sires it is generally accepted that the best measure of an animal's breeding or transmitting value is the kind and production of the progeny the sire begets. However, there are few proven sires that are alive or able to breed when they are proven. Therefore, a large majority of the herd sires selected by our leading dairy cattle breeders are young or at least unproven when selected. These young, unproven bulls are often selected on the basis of individuality alone, or, if the pedigree is used, the breeder does not properly evaluate the pedigree to obtain a good indication of the animal's probable breeding worth. Many breeders have lost faith in pedigrees because they have not properly evaluated the production records of a young sire's ancestry and therefore have been misled by the breeding value of a bull which they thought had a high production pedigree. Rice (3) has used a complicated pedigree index for predicting a sire's transmission of milk production, butterfat test, and butterfat production. The method is too complex for dairymen to use. Putnam and Bowling (1) made a thorough study of using the pedigree in predicting the butterfat test of the daughters of Ayrshire sires. Their method has found use among many Ayrshire breeders.

The United States Department of Agriculture, through the Dairy Herd Improvement Associations in the various states, and the several dairy cattle breed associations have been collecting dairy cattle production records for many years. From these production records, various correction factors, correcting for age of the animal, length of record, and the number of times the cow has been milked daily, have been ascertained. After correcting these production records to a mature, 305-day, twice-daily-milking basis, the indexing of dairy bulls has been determined by comparing their daughters'

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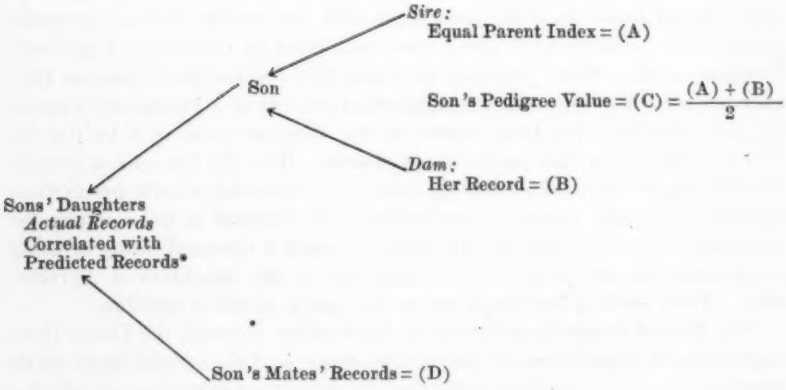
[2] H. C. Dickey, formerly Professor of Dairy Husbandry, Colorado A. & M. College, at present Associate Professor of Dairy Production, University of Vermont. Pedro Labarthe, Graduate Student in Animal Nutrition who aided in accumulating part of the data used in this study.

production with that of the bulls' mates and these indexes published. Thousands of sire indexes thus have been made available to dairy cattle breeders. Unfortunately, these breeders have found little or no practical use for the sire index. This study has been made in an effort to use pedigrees and sire indexes to predict the transmitting ability of young dairy sires.

EXPERIMENTAL

Data for this study were obtained from U.S.D.A. Miscellaneous Publication No. 487, June, 1942. Each Holstein-Friesian proven sire listed in this publication was used and called a "son" in this article. Then the sire and dam of each of these "sons" were determined. If the sire was proven and the dam had a record, the "son" and his pedigree were used. In all, 214 pedigrees were obtained for this study. At first, the following method was used in calculating the predicted milk production, butterfat test, and butterfat production of the "sons' daughters":

METHOD OF PREDICTING BY MEANS OF THE REGULAR PEDIGREE VALUE

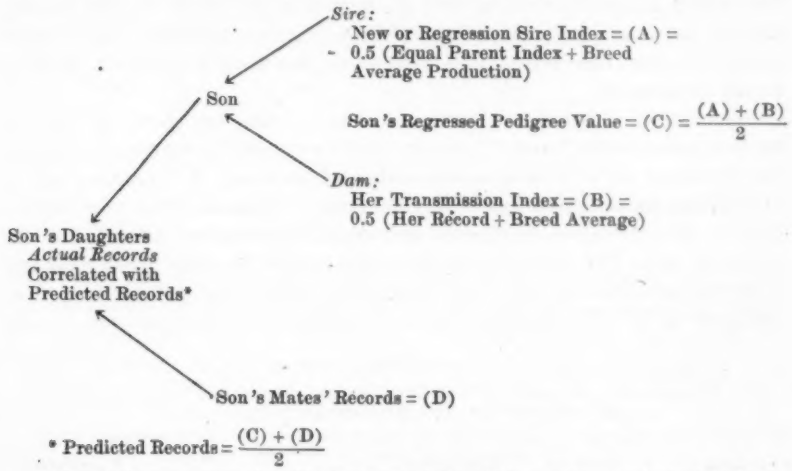


* Predicted Records = $\frac{(C) + (D)}{2}$

All records calculated to a 305-day, twice-daily milking, mature equivalent basis and averaged.

During the time this study was in progress, Rice (2) announced his New or Regression index for proving dairy sires. So it was decided to utilize his findings to obtain a Regressed Pedigree which, in turn, could be used to predict the milk production, butterfat test, and butterfat production of the "sons' daughters" according to the following method:

METHOD OF PREDICTING BY MEANS OF THE REGRESSED PEDIGREE VALUE


Sire:
New or Regression Sire Index = (A) =
0.5 (Equal Parent Index + Breed Average Production)
Son
Son's Regressed Pedigree Value = (C) = (A) + (B) / 2
Dam:
Her Transmission Index = (B) =
0.5 (Her Record + Breed Average)
Son's Daughters
Actual Records
Correlated with
Predicted Records*
Son's Mates' Records = (D)

* Predicted Records = $\frac{(C) + (D)}{2}$

All records are converted to 305-day, twice-daily milking, mature equivalent basis and averaged.

RESULTS

By using the two methods of predicting the milk production, butterfat test, and butterfat production of the "sons' daughters" it was possible not only to make a prediction but also to compare the relative accuracy of the two methods.

Table 1 shows the means, standard errors of estimate, and correlation coefficients for the predicted and actual records of the "sons' daughters" when the regular pedigree value was used for predicting. The predictability of the butterfat test was the highest since its correlation coefficient was 0.630

TABLE 1

Group comparisons for predicted and actual amount of milk and butterfat produced and butterfat test using the regular pedigree value

Item correlated	Kind of record	Number of pedigrees	Mean	Standard error of estimate	Correlation coefficient
			pounds	*pounds*	
Milk	Predicted	214	11,484	1,379	0.565
	Actual	214	10,730		
Butterfat	Predicted	214	404.2	50.7	0.531
	Actual	214	375.0		
			per cent	*per cent*	
Butterfat test	Predicted	214	3.52	0.13	0.630
	Actual	214	3.50		

and the standard error of estimate was only 0.13 per cent. The Babcock Test which is used for determining the butterfat percentage in milk is only slightly more accurate. Most authorities agree that butterfat test is more highly inherited than is total milk production, and should, therefore, be more highly predictable.

The results of using the regressed pedigree value are shown in table 2. In comparing tables 1 and 2 it is seen that the use of the regressed pedigree has increased the correlation coefficients and decreased the standard errors of estimate for milk and butterfat production. There is little or no difference in the correlation coefficients and standard errors of estimate for the butterfat test. The regressed pedigree does predict the milk production and butterfat production of the "sons' daughters" more closely than the regular pedigree value. The standard errors of estimate are 1,316 pounds of milk

TABLE 2

Group comparisons for predicted and actual amount of milk and butterfat produced and butterfat test using the regressed pedigree value

Item correlated	Kind of record	Number of pedigrees	Mean	Standard error of estimate	Correlation coefficient
			pounds	*pounds*	
Milk	Predicted	214	11,427	1,316	0.632
	Actual	214	10,730		
Butterfat	Predicted	214	399.2	47.7	0.606
	Actual	214	375.0		
			per cent	*per cent*	
Butterfat test	Predicted	214	3.49	0.13	0.591
	Actual	214	3.50		

and 47.7 pounds of butterfat when the regressed pedigree is used and 1,379 pounds of milk and 50.7 pounds of butterfat when the regular pedigree is used. The standard error of estimate was 0.13 per cent for butterfat test for each of the two methods used. These results show that the regressed pedigree is a better measure of the transmitting ability of young dairy sires for milk and butterfat production than the regular pedigree value. However, for butterfat test there is little or no difference between the regressed and regular pedigree value for measuring the young dairy sire's transmitting ability.

Although the correlation coefficients are all above 0.53 and therefore rather high, it must be borne in mind that these correlations are based upon the average of all the daughters which each "son" had and not upon each individual daughter. Rice (2) in making daughter-dam correlations found that when individual comparisons were made, that the correlations ranged from 0.289 to 0.322 for milk production and from 0.433 to 0.482 for butterfat test, while with group comparisons the correlations ranged from 0.479 to

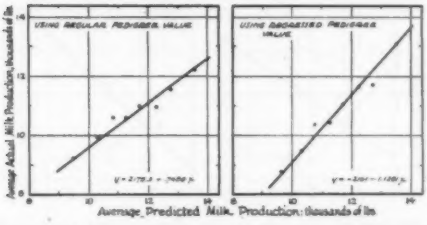

USING REGULAR PEDIGREE VALUE
USING REGRESSED PEDIGREE VALUE
Average Actual Milk Production, thousands of lbs.
Average Predicted Milk Production, thousands of lbs.

FIG. 1. Graph comparing the use of the regular and regressed pedigree value for predicting the milk production of the "sons' daughter."

0.619 for milk production and from 0.446 to 0.610 for butterfat test. The correlations found in tables 1 and 2 are for the most part in the upper range of those found by Rice (2) or higher. This indicates that the use of the "son's" pedigree as well as the "son's" mates' production is necessary in

TABLE 3

Group comparison of predicted and actual milk production of the sons' daughters using the regular pedigree value

When predicted milk production is	Number of pedigrees in each group	Average predicted milk production	Average actual milk production
Less than 10,000	32	9,466	9,230
10,000–10,499	21	10,273	9,953
10,500–10,999	20	10,803	10,594
11,000–11,499	31	11,241	10,596
11,500–11,999	37	11,711	11,007
12,000–12,499	29	12,241	10,942
12,500–12,999	15	12,735	11,547
13,000 or over	29	13,623	12,206

predicting the production and butterfat test of the "sons' daughters." However, in order to obtain a better prediction, some refinement will be necessary. Perhaps by regressing the "son's mates" production with the average breed production, or by applying correction factors to the "son's"

TABLE 4

Group comparison of predicted and actual milk production of the sons' daughters using the regressed pedigree value

When predicted milk production is	Number of pedigrees in each group	Average predicted milk production	Average actual milk production
Less than 10,000	16	9,630	8,685
10,000–10,499	22	10,318	9,503
10,500–10,999	29	10,740	10,365
11,000–11,499	43	11,270	10,413
11,500–11,999	47	11,713	11,059
12,000–12,499	31	12,221	11,632
12,500–12,999	15	12,705	11,699
13,000 or over	11	13,490	13,069

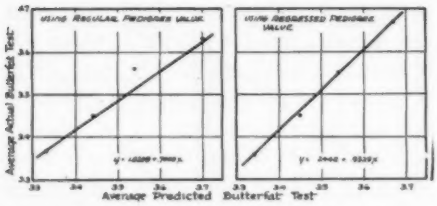


FIG. 2. Graph comparing the use of the regular and regressed pedigree value for predicting the butterfat test of the "sons' daughters."

pedigree at varying levels of production, some refinement can be added to the prediction of milk and butterfat production and butterfat test.

Figure 1 shows that the regressed pedigree is a better method of predicting the actual milk production of the "sons' daughters." The slope of the regression line using the regressed pedigree value is much nearer to one and therefore more desirable than is the regression line using the regular pedigree value. A comparison of the values given in tables 3 and 4 shows that the regular pedigree value predicts the milk production of the "sons' daughters" more closely at the 9,000- and 10,000-pound levels of production, while the use of the regressed pedigree value is a more valuable tool at the 11,000- to 13,000-pound levels of production. It is for these higher levels of production that most dairymen are striving. Therefore, the regressed pedigree value would be the more reliable method to use.

TABLE 5

Group comparison of predicted and actual butterfat test of the sons' daughters using the regular pedigree value

When predicted butterfat test is	Number of pedigrees in each group	Average predicted butterfat test	Average actual butterfat test
Less than 3.40	35	3.33	3.37
3.40–3.49	60	3.44	3.45
3.50–3.59	62	3.54	3.56
3.60 or over	57	3.70	3.63

TABLE 6

Group comparison of predicted and actual butterfat test of the sons' daughters using the regressed pedigree value

When predicted butterfat test is	Number of pedigrees in each group	Average predicted butterfat test	Average actual butterfat test
Less than 3.40	28	3.34	3.36
3.40–3.49	88	3.45	3.45
3.50–3.59	66	3.54	3.55
3.60 or over	32	3.67	3.67

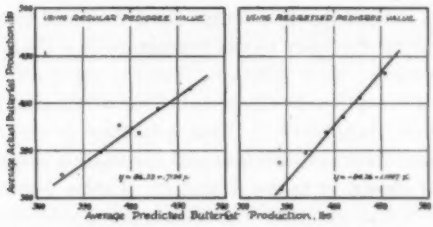


FIG. 3. Graph comparing the use of the regular and regressed pedigree value for predicting the butterfat production of the "sons' daughters."

For predicting the butterfat test of the "sons' daughters," figure 2 shows that the regressed pedigree value is a better measure than is the regular pedigree value. The slope of the regression line using the regressed pedigree value shows that this is a better method than is the regular pedigree. An

TABLE 7

Group comparison of predicted and actual butterfat production of the sons' daughters using the regular pedigree value

When predicted butterfat production is	Number of pedigrees in each group	Average predicted butterfat production	Average actual butterfat production
Less than 360	34	337.0	325.1
360–379	29	367.8	349.5
380–399	34	388.9	376.7
400–419	35	409.5	367.9
420–439	33	429.0	394.0
440 or over	49	462.8	415.2

examination of tables 5 and 6 also shows that the regressed pedigree value is a better measure of the butterfat test than is the regular pedigree value at all levels of butterfat test studied.

Figure 3 indicates the regression lines which show how well the regular pedigree value and the regressed pedigree value predict the actual butterfat production of the "sons' daughters." When the regressed pedigree value

TABLE 8

Group comparison of predicted and actual butterfat production of the sons' daughters using the regressed pedigree value

When predicted butterfat production is	Number of pedigrees in each group	Average predicted butterfat production	Average actual butterfat production
Less than 360	24	344.3	310.3
360–379	35	369.3	348.1
380–399	48	389.6	367.9
400–419	48	409.7	384.2
420–439	35	427.1	405.6
440 or over	24	454.4	431.6

is used, the points indicating the average predicted and actual butterfat production are considerably closer to the regression line than when the regular pedigree value is used. This indicates that the regressed pedigree value is more useful than the regular pedigree value for predicting the butterfat production of the "sons' daughters." This indication is also borne out by comparing the standard errors of estimate and correlation coefficients for butterfat production as shown in tables 1 and 2. Tables 7 and 8 show that the regular pedigree value predicts the butterfat production of the "sons' daughters" more closely at lower levels of production, while the regressed pedigree value gives a better measure at the intermediate and higher levels of production.

CONCLUSIONS

Two methods of predicting the transmitting ability of young dairy sires have been shown. The regressed pedigree value is superior to the regular pedigree value in predicting the transmission of milk production and butterfat production.

The two methods are about equal in predicting the transmission of butterfat test.

ACKNOWLEDGMENT

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A COMPARISON OF VARIOUS MODIFICATIONS OF THE BABCOCK TEST FOR THE TESTING OF HOMOGENIZED MILK*

G. M. TROUT AND P. S. LUCAS
Michigan Agricultural Experiment Station, East Lansing

Research workers are not in perfect agreement as to the reliability of the Babcock test in determining the percentage of fat in homogenized milk as compared to the same milk not homogenized.

Bishop and Murphy (6) stated "Although the fat cannot be separated out by the centrifuge separator there is very little difference in the Babcock test. We have always found that duplicate samples of homogenized and unhomogenized milk tested nearly the same, the homogenized testing usually about two-thirds per cent lower." Later they stated that the readings were within the limits of the possible variation of two samples of the same milk. Likewise, Doan and Swope (15) observed that homogenization, even at high pressure, had but little influence on the Babcock test. On the other hand, Hollingsworth (18) and Hudon (20) reported that dealers had realized that milk after homogenization would not yield the same percentage of fat by the Babcock test as before homogenization. They explained this on the basis of a greater percentage of residual fat remaining in the homogenized milk test, which may amount to two- or three-tenths of 1 per cent.

Contrary to this observation, Halloran and Trout (17) found that homogenization appeared to have no effect on the Babcock test of whole milk. The United States Department of Agriculture (33) reported that the Babcock test failed to recover as much fat in homogenized milk as it did in unhomogenized milk and for that reason, when the test was used to determine the butterfat content of milk, the fat content of homogenized milk would appear slightly lower than for the same milk prior to homogenization. The data of Babcock (2, 3) supports this belief. He found that the Babcock test failed to recover as much of the fat in homogenized milk as it did in unhomogenized milk, the test averaging 0.1 per cent lower in the homogenized milk than in the same milk not homogenized.

In general, the data of Hood and White (19) are in agreement with those of Babcock. While their data definitely indicated that homogenization tended to lower the Babcock fat test, they concluded that there should be no difficulty in having the test of homogenized milk check to within 0.1 per cent of the nonhomogenized milk when the operation of the Babcock test was carefully carried out.

Tracy (27, 28) encountered no great difficulty in securing tests comparable to the results secured on unhomogenized samples, but later (29) reported

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that homogenized milk showed a tendency to test lower (about 0.1 per cent) than nonhomogenized milk. Likewise Tretsven (30) concluded that homogenization tended to lower the Babcock fat test of milk.

This conclusion was supported by Doan (13) who stated that the usual Babcock test gave somewhat lower readings with homogenized milk but in no case should the readings be more than 0.1 point low. Later, Doan, Josephson and Adams (14) and Doan (12) found that the modified Babcock test was practically as accurate for homogenized milk as the unmodified test was for unhomogenized milk.

Brueckner (7), in answer to an inquiry concerning the Babcock test of homogenized milk, stated that when milk was homogenized at 2,000 to 3,000 pounds pressure the Babcock test would be about 0.1 to 0.15 per cent lower than before homogenizing.

These differences between the Babcock test of homogenized and unhomogenized milk may be due in part to one or more of several factors such as (a) the specific gravity of the acid used; (b) the volume of acid; (c) the maximum heat of reaction obtained; (d) the length of the time of shaking the acid-milk mixture; (e) the completeness of digestion and (f) the amount of foreign matter in, or at the base of the fat column.

Persons testing homogenized milk early recognized that clear fat columns supported by a clear water column were obtained with difficulty. Burr (8) early pointed out that in Gerber tests of homogenized milk upon prolonged centrifuging in unheated centrifuges, plugs were found at the contact zone between the fat column and the sulphuric acid mixture which interfered with accurate reading of the test. Later Bishop and Murphy (6) found that it was more difficult to get a clear reading with the Babcock test on homogenized milk than on unhomogenized milk.

Bailey (5) in his extensive studies on the Babcock test observed that casein was dissolved with more difficulty when fat was homogenized into the solution. Data indicated that as the volume of acid was increased a lesser percentage of insoluble organic material remained in the mixture.

Trout and coworkers (31, 32) observed a plug of foreign material varying in thickness from 1/32 to 1/64 of an inch at the base of the fat column of Babcock tests made on homogenized milk. This plug of foreign material resembled the curdy test sometimes encountered in testing nonhomogenized milk. If the fat column receded during tempering, this thin, tenacious plug usually assumed a perpendicular position along the side of the graduated column, displacing some of the fat and causing a higher fat reading. When more or stronger acid was used in an attempt to digest completely all the proteins of homogenized milk, and thus eliminate the plug, a burned test invariably resulted in which little assurance as to accuracy of the test could be placed. By varying the strength of acid and the temperatures of the acid and milk and by using care in manipulation of the test good readable tests were made, but the plug was usually present to a certain extent.

Hood and White (19) also noted this plug of material at the base of the fat column of homogenized milk tests and believed that it should be included in reading the test or the test would be this much lower than that of the non-homogenized milk.

Tracy (29) noted that it was more difficult to test homogenized milk by the Babcock method because of the tendency for charred and greyish material to accumulate in the fat column.

Consequently, research workers have modified the Babcock test slightly in testing homogenized milk in order to secure a more perfect test. Trout, Halloran and Gould (32) used sulphuric acid having a specific gravity of 1.815 to 1.820 and exercised greater care in mixing the milk and acid with the result that clearer more readable tests were obtained.

Tracy (27, 28) secured best results when the acid and milk were both at room temperature (70° F.) and when the acid was added a little at a time, shaking after each addition, and then shaking well after all the acid had been added. He stated, "Usually slightly less (about 1.5 ml.) acid was needed for the homogenized milk as it tended to char when the regular amount was used. As the pressure of homogenization was increased more difficulty from charring was experienced." Later Tracy (29) reported securing best results when the amount of acid was reduced only slightly, to about 17 ml., and was added in three portions, shaking well after each addition. He advised that the acid should be at a temperature of about 72° F. and the milk at 50–55° F.

Ruehe (26) obtained satisfactory results by tempering milk and acid to 70° F. and using 16 ml. of regular sulphuric acid, adding it in five portions. Roadhouse and Henderson (25) advocated the use of 16.0 to 16.5 ml. of acid, adding it in at least three portions with considerable rotation of the bottle between the additions of acid and shaking "the bottles as soon as the water has been added after the first and second centrifugings." Such modifications, they believed, would give results that should not be more than 0.1 per cent below the test of the same milk unhomogenized.

Hood and White (19) secured best results in testing homogenized milk by tempering both the milk and acid to 40° to 50° F. and by shaking thoroughly the separated fat with the acid-plasma-water mixture after each centrifuging. By this technique they eliminated the plug entirely in the great majority of cases.

Doan (13) observed that by adding 16.5 ml. of sulphuric acid in three portions, mixing the acid and milk with a rotary motion after each addition, and remixing the bottle contents after the first addition of water, a clear fat column was obtained. This also aided in preventing the plugs or discs which sometimes formed at the base of the neck of the bottles. Later, Doan, Josephson and Adams (14) used essentially this same modification except that 17 ml. of sulphuric acid was used instead of 16.5 ml. with virtually the same results.

Brueckner (7) observed greater difficulty in getting a clear test when testing homogenized milk than when testing nonhomogenized milk under the same conditions. In overcoming this difficulty he recognized the value of reducing the amount of acid used in the Babcock test by about 1.5 ml., adding the acid in 3 or 4 portions, and shaking the milk and acid after each addition. He found that by adding a mixture of alcohol and water in place of the last hot water added during the centrifuging gave a clear fat column. The water-alcohol mixture was made by mixing 1 part by weight of alcohol to 1.4 parts by weight of water. He stated that tests obtained by using the water-alcohol mixture agreed with the Mojonnier or official method for determining fat in milk.

In overcoming the difficulty of testing homogenized milk, Crowe (11) advised using sulphuric acid having a specific gravity of 1.815 to 1.820 and adding it in small portions at 60 to 70° F. or lower, then mixing the bottle contents after the first centrifuging.

Marquardt (24) eliminated the sediment under the Babcock fat column of homogenized milk by adding the acid in three portions, shaking well after each addition and then by thoroughly shaking the samples after (a) the first centrifuging, (b) after half the first addition of water, (c) after completing the addition of water prior to the second centrifuging and (d) again after the second centrifuging before the final addition of water. Such a procedure yielded clear fat columns.

Coulter (10) in answer to an inquiry concerning the Babcock test of homogenized milk in which the fat columns were not clear and the readings too low replied in part that he had secured fairly satisfactory results in their laboratory using Marquardt's (24) modification of the Babcock method.

Judkins (23) found that the modified Babcock tests used in milk plants resulted in too many imperfect tests and, therefore, developed a more satisfactory modification of the Babcock procedure for testing homogenized milk, involving a 10-minute first centrifuging period the details of which are included in table 1.

Whitaker (34) reported also that it was advisable in testing homogenized milk to centrifuge the test for 10 minutes during the first centrifuging period rather than the customary 5 minutes. Other modifications involved the use of slightly less acid, depending upon its strength, than normally required and mixing the acid-serum-water solution thoroughly after each addition of water.

Another method (1) advocated, for testing homogenized milk, the use of 17.5 ml. of acid added in two equal portions with prolonged agitation of the milk and acid after each addition, followed by a 5-minute digestive period before centrifuging. The total centrifuging time advocated was 19 minutes with additions of water and centrifuging period as follows:

> At the end of the first 5 minutes of centrifuging, stop the centrifuge and add about ½ the amount of water normally added in a regular Babcock test. This water added to

the sulphuric acid milk mixture should be a temperature of 140° F. or higher. Centrifuge again for 5 min. and again add water to bring the mixture about ¼th of an inch from the base of the neck of the Babcock test bottle. Centrifuge for 5 more minutes, stop, and add enough water to bring the butterfat to the required position in the neck of the bottle as in the normal Babcock test. Then, centrifuge for 4 minutes and remove the sample from the centrifuge.

The charred material at the base of the fat column is not confined entirely to Babcock fat tests of homogenized milk. Babcock (4), in devising the test bearing his name, noted the plug of foreign material and made recommendations for its elimination. In overcoming the intermingling of caseous material with the fat which prevented accurate reading, he advised:

It sometimes happens in testing buttermilk, that is quite sour, that the addition of water to bring the fat into the neck of the bottle where it can be measured, causes a precipitate of casein or other matter which mingles with the fat and prevents an accurate reading. This difficulty has also occurred a few times with milk that had stood a considerable time before testing: it may be entirely avoided by filling the bottles with a hot mixture of equal parts of sulphuric acid and water instead of water alone.

It must be remembered that the difficulty to which Babcock referred occurred on nonhomogenized milk.

Hunziker (21), studying the estimation of fat in unsweetened evaporated milk, observed black sediment and charred masses in the necks of the test bottles which interfered with accurate reading. The difficulty reported was similar in many respects to that encountered in the Babcock testing of homogenized milk. He stated: "In the case of the Babcock test of this evaporated milk, clear tests are the exception rather than the rule. The repeated appearance of black sediment and flocculent charred masses in the necks of the test bottles seriously interferes with the accuracy of the reading and introduces an element of doubt as to the reliability of the tests." He (22) believed that the samples under investigation were not homogenized, as homogenization was not a part of processing evaporated milk at that time. However, the following description given by him (21) in 1909 might well apply to homogenized milk tests by the regular Babcock method:

A large number of tests are not clear: either the fat is mixed with charred masses, or there is a layer of curdy sediment at the bottom of the fat column, or both. Tests of this kind are very unsatisfactory, as they leave the operator in doubt as to how to read the fat column. He does not know whether the black and curdy layer should be included or not. The result is that the reading of the fat column is largely guess work, and the correct interpretation of the results is accidental rather than regular.

The cause of the precipitation of these curdy and charred masses was, therefore, carefully studied. It was observed that the mixture of diluted condensed milk and acid remained in apparent solution up to the time the hot water is added after the first whirling. After the addition of hot water the precipitation seemed to appear. Though the tests were made with milk from the same can, and as far as noticeable under identical conditions as to degree of dilution, amount of acid, amount of milk, temperature of milk, acid

and water, etc., there was no uniformity in the clearness of the tests. Some tests showed a very heavy precipitation, the charred masses making accurate reading impossible; in some tests there was a deep layer of curdy sediment at the bottom of the fat column; in others this layer was slight, and a few tests were entirely clear. On the average about five out of every six tests were charred and contained sediment and some were so bad that they could not be read at all.

This difficulty in the Babcock fat test of evaporated milk was finally overcome, not by varying the amount and strength of the sulphuric acid, nor by the addition of solvents as borax or potassium hydrate before adding the acid, nor by allowing the tests to stand 24 hours, but by the addition to the test bottles of hot dilute sulphuric acid (one part acid to one part water) in the place of hot water after the first whirling—a method similar to that suggested earlier by Babcock (4) in order to render the fat columns clear.

In view of the many modifications of the Babcock test for the specific purpose of preventing the formation of plugs at the base of the fat columns of homogenized milk, it seemed desirable to compare the various tests as to their reliability and as to their efficiency in overcoming the defect. The various modifications of the Babcock test advocated for homogenized milk which were studied are presented under procedure.

PRINCIPLE UNDERLYING MODIFICATION OF THE BABCOCK TEST FOR HOMOGENIZED MILK

The principle underlying most of the modifications of the Babcock test for homogenized milk seems to be that of keeping the temperature of the acid-milk mixture low without sacrificing the strength of the acid. The maximum temperature obtained in mixtures of sulphuric acid and milk is dependent upon several factors such as (a) the initial temperatures of the acid and milk; (b) the specific gravity of the acid; (c) the total volume of acid used; (d) the portions of acid added and mixed with the milk at one time; and (e) the rate of mixing the acid and milk. The effect of some of these factors on the temperature of the acid-milk mixture is shown in figures 1 and 2. Observations on many tests indicated that best results were secured when the temperature of the acid-milk mixture was kept relatively low, but the digestive action of the acid was maintained at a high level through the use of a sufficient volume of high specific gravity acid.

In many respects the testing of homogenized milk by the Babcock method is analogous to that of cream. Greater difficulty in securing perfect tests are generally encountered in testing a 40 per cent cream than a 20 per cent cream. The 40 per cent cream, having a greater total fat-globule surface area than 20 per cent cream, apparently has more adsorbed casein than the 20 per cent cream which, unless precautions are taken to prolong the action of the acid, may not be completely digested and may appear as a plug at the base of the fat column. So it appears to be with homogenized milk.

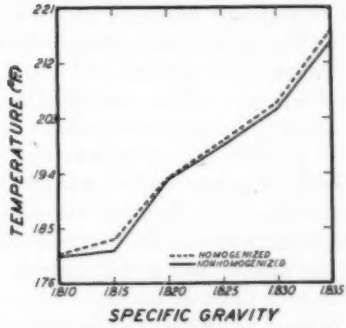

FIG. 1. The temperature of acid-milk mixtures when 16 ml. of sulphuric acid of different specific gravities was used.

Wiegner (35) calculated that the fat globules of nonhomogenized milk adsorbed about 2 per cent of the casein of milk whereas those of homogenized milk had adsorbed about 25 per cent. Corbett and Tracy (9) observed that homogenization encloses the fat globule in a protective, casein-type membrane. This caseous membrane seems to be the constituent in association with homogenized fat which causes difficulty in securing fat tests of the homogenized product free from insoluble organic matter. Bailey's (5) early work showed that when equal volumes of acid were employed in testing a 3-per cent casein solution with and without 3.5 per cent fat homogenized into it that considerably more insoluble organic material was present in the homogenized fat-casein series than in the casein series alone. By increasing the sulphuric acid from 10 to 20 ml. the percentage increase in insoluble organic matter in the homogenized fat-casein mixture over that of the casein series was decreased from approximately 560 per cent to 50 per cent.

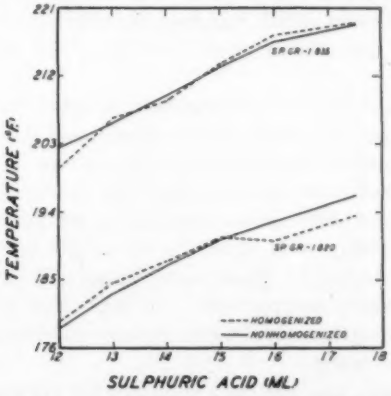

FIG. 2. The temperature of acid-milk mixtures when different volumes of sulphuric acid were used.

The complete digestion of this adsorbed layer on the homogenized fat globules without burning the nonadsorbed casein apparently is necessary if good Babcock tests of homogenized milk are to be had. Attainment of this balance in testing of homogenized milk is not easy, but may be approached if the difficulties to be encountered are appreciated. Since the casein particles adsorbed on the fat globules cannot be completely surrounded by the sulphuric acid, being protected in part and for a brief time at least by the fat itself, it will be digested more slowly than the free nonadsorbed casein. On the other hand, the free, nonadsorbed casein, representing 75 per cent of the total, will be digested too rapidly and a charred test will result. Consequently, to secure satisfactory Babcock fat tests of homogenized milk, digestion of part of the casein must be retarded while that of the remainder must be prolonged.

PROCEDURE

The tests studied and reported herein were modifications of the Babcock test devised primarily for the testing of homogenized milk. All the tests made use of the means and chemicals ordinarily available in a milk plant and required no elaborate technique for their operation. Because of their relative simplicity these tests are usually acceptable to the dairy industry as practical methods.

The many tests suggested for the determination of fat in homogenized milk indicate in itself that some of them must be more or less unsatisfactory under varying conditions of testing. Undoubtedly, when every minute detail of testing procedure as prescribed is followed precisely, reasonable assurance of good results may be had. Unfortunately, such exactness may not always be observed in practical work.

Twelve modified Babcock-test methods were studied. For the sake of convenience these are given in table 1. Each test was run in duplicate in Babcock milk test bottles which had been recalibrated for accuracy. Any bottles showing 0.1 per cent or more variation from exact accuracy were discarded.

Observations made on the duplicate tests included the percentage of fat, color of the fat column, the clarity of fat column, and the homogeneity of the acid-serum-water mixture supporting the fat column. Other factors, however, which might influence their acceptability to the plant operator were noted. These included difficulties in regulating temperatures and strength of the acid used, rapidity with which the test might be made and the difficulty of technique involved. These latter factors affect the practicability of any test in laboratory control work. In order that both the advantages and disadvantages of each method might be fully observed, all tests were run and completed individually.

The Mojonnier test was used as a standard for accuracy. However, instead of using a volumetrically measured approximate ten-gram portion, the

TABLE 1

Various modifications of the Babcock test for homogenized milk

Test No.	Authority	Specific recommendations as to								Time of centrifuging
		Acid					Milk	Mixing the acid and the milk		
		Sp. gr.	Temp. (° F.)	Amount (ml.)	Portions added		Temp. (° F.)	Before centrifuging	After centrifuging	
					No.	Ml.*				
1	Trout, Halloran and Gould (31, 32)	1.815–1.820						Careful, prolonged with a smooth rotary motion		
2	Tracy (27, 28)		70	16	Small	4–4–4–4	70	After each addition and well after all acid added		
3	Tracy (29)		72	17	Three	6–6–5	50–55	Well, after each addition		
4	Ruehe (26)		70	16	Five	3–3–3–3–4	70	After each addition		
5	Roadhouse and Henderson (25)			16–16.5	Three	6–5–5.5		Well, after each addition	After the addition of water following first and second centrifugings	
6	Hood and White (19)		40–50				40–50		Shaking separated fat with acid-serum-water mixture after each centrifuging	
7	Doan (13)			16.5	Three	6–5–5.5		After each addition	After the first addition of water	
8	Doan, Josephson and Adams (14), Doan (12)		Room	17	Three	6–6–5	Room	After each addition	After the first addition of water	

TABLE 1 (*Continued*)

Test No.	Authority	Specific recommendations as to								Time of centrifuging
		Acid					Milk	Mixing the acid and the milk		
		Sp. gr.	Temp. (° F.)	Amount (ml.)	Portions added		Temp. (° F.)	Before centrifuging	After centrifuging	
					No.	Ml.*				
9	Marquardt (24)				Three	6–6–5.5		After each addition	After the a. first centrifuging b. addition of one-half the first volume of water c. final addition of the first volume of water d. second centrifuging	
10	Whitaker (34)		Room	Slightly less than 17.5	Three to four	7–3–3–4.5	Room	Thorough mixing between each addition	Diluted acid-milk mixture should be mixed well before filling into neck	First whirling ten min.
11	Anonymous (1)			17.5	Two	9–8.5		After each addition 2 or 3 times as long as in regular Babcock procedure. Hold 5 min. before centrifuging	After first 5 min. Then add ½ amount water and centrifuge 5 min.; add remainder of water; centrifuge 5 min.; add final water; centrifuge 4 min.	Total 19 min.
12	Judkins (23)	1.82–1.83	60	15	Three to four		60	No red tinge should be visible at time bottles are placed in centrifuge	Add hot water to ½–⅝″ below the neck; centrifuge 2 min.; shake; allow fat to coalesce then fill up into neck	First whirling ten min.

* Specific volumes were not recommended; volumes given were amounts used in studies.

samples previously tempered to 70° F., tested in duplicate, were weighed carefully on a chemical balance in each case directly into fat-extraction flask. In addition, the various tests on the homogenized product were compared to those of the regular Babcock method on nonhomogenized milk.

Five series each of higher and of lower testing milk, before and after homogenization, were tested in duplicate by the modified Babcock procedures, as well as by the regular Babcock and Mojonnier methods. The unhomogenized samples were taken from the vat after pasteurization prior to homogenization. The milk had been kept thoroughly mixed during pasteurization and homogenization so as to insure uniform fat distribution. The homogenized samples were taken from the cooled bottled product after homogenization was well under way. Mojonnier tests showed that the nonhomogenized and homogenized samples contained similar percentages of fat.

Homogenization was done by means of a 500-gallon-per-hour viscolizer at 2500 pounds pressure at 130° F. following pasteurization. The collected samples were adequately cooled, stored and tested as rapidly as time would permit. In testing the samples according to the various modifications, pipetting of the 17.5 ml. portions of milk into the test bottles was done for all tests at one time to assure correct sampling. This was done after the milk had been tempered at 70° F. for two hours. These charged test bottles were then stored at 40° F. until the tests were made.

The techniques of the tests were followed as closely as possible. When the acid was added in several portions this was done by means of a burette so accurate additions could be made. The modifications were run in order to check the method as a possible one which would eliminate the plug formation and at the same time check closely with an official method. A series of nonhomogenized milk was tested by each of the eleven methods to see whether the plug formation might be due to homogenization or to the method used.

When char, crystallized fat, curd or foreign material was encountered at the base of the fat column, this plug was included in the reading as part of the fat. Early observations indicated that it was difficult to eliminate this often feathery material from the fat reading and that its inclusion was essential if the fat percentage were to compare favorably with the official test of that of the nonhomogenized product.

RESULTS

Accuracy of the various modified Babcock tests. Five series each of lower testing and of higher testing milk, nonhomogenized and homogenized, were tested for fat by the regular Babcock method, by the Mojonnier method, and by 10 modifications of the Babcock method. One series of lower and of higher testing milk was tested also by an additional modified method. The percentages of fat, the deviations of these percentages from the Mojonnier tests and the deviations from the Babcock tests of the nonhomogenized samples

are presented in tables 2, 3, and 4, respectively. These data show not only considerable variation between the modified methods in the average deviation both from the Mojonnier tests of the homogenized milk and the Babcock tests of the nonhomogenized sample, but also within the specific tests themselves. In general, the data show a greater number of the methods reliable

TABLE 2

The average percentages of fat in homogenized milk as determined by various modifications of the Babcock method

Type of milk	Method	Percentage fat (ave. 2 trials each) in series					
		A	B	C	D	E	Mean
Lower testing:							
Not homogenized	Babcock	3.70	3.60	3.55	3.93	3.80	3.72
	Mojonnier	3.70	3.65	3.54	3.95	3.78	3.73
Homogenized	Babcock	3.63	3.55	3.44	3.85	3.62	3.62
	Mojonnier	3.70	3.65	3.54	3.94	3.77	3.72
	Modified Babcock No. 1	3.60	3.48	3.50	3.93	3.70	3.64
	2	3.68	3.55	3.25	3.85	3.68	3.60
	3	3.55	3.60	3.35	3.80	3.70	3.60
	4	3.70	3.50	3.40	3.83	3.70	3.63
	5	3.70	3.60	3.45	3.88	3.70	3.67
	6	3.55	3.50	3.35	3.80	3.65	3.57
	7	3.68	3.60	3.38	3.88	3.70	3.65
	8	3.70	3.60	3.48	3.95	3.68	3.68
	9	3.58	3.55	3.48	3.88	3.70	3.64
	10	3.70	3.60	3.43	3.90	3.73	3.67
	11	.....	.....	.....	.....	3.75	.....
Higher testing:							
Not homogenized	Babcock	4.48	4.50	4.40	4.55	5.00	4.59
	Mojonnier	4.43	4.45	4.37	4.56	4.99	4.56
Homogenized	Babcock	4.40	4.38	4.30	4.50	5.00	4.52
	Mojonnier	4.46	4.47	4.37	4.58	4.98	4.57
	Modified Babcock No. 1	4.40	4.35	4.25	4.35	4.88	4.45
	2	4.33	4.40	4.33	4.43	4.83	4.46
	3	4.45	4.40	4.30	4.50	4.88	4.51
	4	4.35	4.38	4.25	4.43	4.83	4.45
	5	4.35	4.40	4.33	4.55	4.80	4.49
	6	4.30	4.38	4.30	4.33	5.00	4.46
	7	4.40	4.38	4.30	4.48	4.90	4.49
	8	4.43	4.40	4.33	4.50	4.80	4.49
	9	4.43	4.38	4.28	4.43	4.83	4.45
	10	.....	4.40	4.38	4.50	4.90	4.54
	11	.....	.....	.....	.....	4.95	.....

within the 0.1 per cent limit of accuracy when applied to lower than when applied to higher testing milk. However, methods numbers 5, 7, 8 and 10 seemed to yield tests consistently closer to those of the Mojonnier and to those of the regular Babcock test on nonhomogenized milk than did the other tests. These tests have in common (a) the use of the approximate or full amount of acid required of the Babcock test, (b) the addition of the acid in at least three portions and (c) the remixing of the acid-plasma-water mix-

ture after centrifuging and following the addition of water. Of the five above-mentioned methods, numbers 8 and 10 or 11 seemed to yield tests consistently closer to the standard than did the others. Nothing seemed to be gained by adding the acid in five portions in the amounts used as in number 4. The method employing the full amount of acid, 17.5 ml., but of a specific gravity of 1.810 to 1.820 (method No. 1) yielded tests within the recognized accuracy of 0.1 per cent when used on lower testing milk, but was

TABLE 3

Deviations of modified Babcock tests of homogenized milk from those of the Mojonnier methods

Modified Babcock method (No.*)	Deviations of fat tests of homogenized milk of series					
	A	B	C	D	E	Mean
From Mojonnier tests—Lower testing milk						
1	−0.10	−0.17	−0.04	−0.01	−0.07	−0.08
2	−0.02	−0.10	−0.29	−0.09	−0.09	−0.12
3	−0.15	−0.05	−0.19	−0.14	−0.07	−0.12
4	0.00	−0.15	−0.14	−0.11	−0.07	−0.09
5	0.00	−0.05	−0.09	−0.06	−0.07	−0.05
6	−0.15	−0.15	−0.19	−0.14	−0.12	−0.15
7	−0.02	−0.05	−0.16	−0.06	−0.07	−0.07
8	0.00	−0.05	−0.06	+0.01	−0.09	−0.04
9	−0.12	−0.10	−0.06	−0.06	−0.07	−0.08
10	0.00	−0.15	−0.11	−0.04	−0.04	−0.05
11	.....	.....	.....	.....	−0.02	.....
From Mojonnier tests—Higher testing milk						
1	−0.06	−0.12	−0.12	−0.23	−0.10	−0.12
2	−0.13	−0.07	−0.04	−0.15	−0.15	−0.11
3	−0.01	−0.07	−0.07	−0.08	−0.10	−0.06
4	−0.11	−0.09	−0.12	−0.15	−0.15	−0.12
5	−0.11	−0.07	−0.04	−0.03	−0.18	−0.08
6	−0.16	−0.09	−0.07	−0.25	+0.02	−0.11
7	−0.06	−0.09	−0.07	−0.10	−0.08	−0.08
8	−0.03	−0.07	−0.04	−0.08	−0.18	−0.08
9	−0.03	−0.09	−0.09	−0.15	−0.15	−0.12
10	.....	−0.07	+0.01	−0.08	−0.08	−0.03
11	.....	.....	.....	.....	−0.03	.....

* See table 1 for details of procedure.

not so accurate in testing higher fat milk. On the other hand, the method involving low temperature milk (No. 3) yielded more accurate tests on high fat milk than it did on low fat milk.

In addition to accuracy, other factors, such as appearance of the fat column including its supporting medium and the ease of manipulation must be considered.

The appearance of the tests. The modified Babcock methods studied yielded fat columns which often varied markedly in appearance. Data of one series, representative of the studies, are presented in table 5. Here it will be seen that the foreign material frequently present in, or at the base

of the fat column of Babcock tests of homogenized milk may be either curdy, grey, or black charred and may be much or little. Also, the foreign material may be compact and tenacious or loose and scattered. Furthermore, the fat itself may be cloudy or oily clear, and may range from white to a golden color. Certain conditions of the foreign material—large amount, dark color, and scattered distribution into the fat or supporting water column—are such as to cause the operator to have little confidence in the accuracy of the test. While each of the modified methods studied yielded fat columns compara-

TABLE 4

Deviations of modified Babcock tests of homogenized milk from those of the regular Babcock method on the same milk not homogenized

Modified Babcock method (No.*)	Deviations of fat tests of homogenized milk of series					
	A	B	C	D	E	Mean
From Babcock tests of nonhomogenized sample—Lower testing milk						
1	−0.10	−0.12	−0.05	0.00	−0.10	−0.08
2	−0.02	−0.05	−0.30	−0.08	−0.12	−0.12
3	−0.15	0.00	−0.20	−0.13	−0.10	−0.12
4	0.00	−0.10	−0.15	−0.10	−0.10	−0.09
5	0.00	0.00	−0.10	−0.05	−0.10	−0.05
6	−0.15	−0.10	−0.20	−0.13	−0.15	−0.15
7	−0.02	0.00	−0.17	−0.05	−0.10	−0.07
8	0.00	0.00	−0.07	+0.02	−0.12	−0.04
9	−0.12	−0.05	−0.07	−0.05	−0.10	−0.08
10	0.00	0.00	−0.12	−0.03	−0.07	−0.05
11	.....	.....	.....	.....	−0.05	.....
From Babcock tests of nonhomogenized sample—Higher testing milk						
1	−0.08	−0.15	−0.15	−0.20	−0.12	−0.14
2	−0.15	−0.10	−0.07	−0.12	−0.17	−0.13
3	−0.03	−0.10	−0.10	−0.05	−0.12	−0.08
4	−0.13	−0.12	−0.15	−0.12	−0.17	−0.14
5	−0.13	−0.10	−0.07	−0.00	−0.20	−0.10
6	−0.18	−0.12	−0.10	−0.22	−0.00	−0.13
7	−0.08	−0.12	−0.10	−0.07	−0.10	−0.10
8	−0.05	−0.10	−0.07	−0.05	−0.20	−0.10
9	−0.05	−0.12	−0.12	−0.12	−0.17	−0.14
10	.....	−0.10	−0.02	−0.05	−0.10	−0.05
11	.....	.....	.....	.....	−0.05	.....

* See table 1 for details of procedure.

tively free of the foreign material in contrast to that obtained when using the regular Babcock method, nevertheless some foreign material was invariably present. Application of each of the modified methods to nonhomogenized milk showed conclusively that the method was not a contributor to the foreign material since each of the methods yielded perfect tests on the nonhomogenized product. In general, methods 1, 3, and 6, which employed either weak acid, a low temperature milk or acid, or both, yielded pale to white fat columns, which were generally cloudy, but remarkably free from sediment. On the other hand methods 8 and 10 more consistently yielded

clear, golden fat columns with a minimum of foreign material than did the remaining methods.

Difficulties of techniques of the various modified Babcock procedures. No especial difficulty was encountered in the technique of any of the methods studied. However, some methods were found to be more tedious and time-consuming than others. Excepting methods No. 1 and 6, all the procedures involved adding the acid in from two to five portions, the latter of which involved considerable time. Method No. 1 was the only one specifying a specific gravity of acid differing from that of the regular Babcock procedure. This required standardizing a special strength acid. Methods 3 and 6 made

TABLE 5

The appearance of fat tests made on homogenized milk by various modifications of the Babcock procedure. (Evaluation based upon 10 tests by each method)

Modified Babcock test (No.)	The character of the fat column as to				
	Color	Clarity	Foreign material		General appearance
			Amount	Color	
1	Light	Sl. cloudy	$\frac{1}{64}$" to $\frac{1}{32}$"	Grey	Pale, feathery
2	Normal	Clear	$\frac{1}{64}$" to feathery	Grey to brown	Good
3	Light	Sl. cloudy	$\frac{1}{64}$" to v. sl. feathery	Grey to brown	Sl. light, but good
4	Sl. light	Sl. cloudy	$\frac{1}{64}$" to feathery	Grey, clear	Good
5	Normal	V. clear	Trace; feathery	Light brown	Excellent
6	Light	Sl. cloudy	$\frac{1}{64}$" to $\frac{1}{32}$"	Grey, curdy	Pale, but good
7	Normal	Clear	Trace to feathery	Light brown	Very good to excellent
8	Normal	Clear	Trace to sl. feathery	V. light brown	Excellent
9	Normal	Clear	Feathery	Light brown to dark	Excellent
10*	Normal	Clear	V. sl. feathery	Dark	Excellent
11*	Normal	Clear	V. sl. feathery	Dark	Excellent

* Two trials.

use of lower than room temperatures which necessitated special attention. Methods, 5, 6, 7, 8, 9, 10, 11 and 12 required remixing the acid-serum-water mixture after each centrifuging. Some methods, No. 9 and 11, recommended adding the first volume of water in two parts, shaking the mixture after each addition. Methods 10 and 12 called for a 10-minute first centrifuging in place of the customary 5 minutes, whereas the total centrifuging time of method 11 was 19 minutes, eleven minutes longer than that of the regular Babcock method. No advantage seemed to be gained by increasing the number of additions of acid beyond three. Likewise prolonged manipulation of the sample by adding the water in several portions seemed unnecessary. With these few exceptions the modified procedures were not tedious to carry out.

Influence of various fat-column-supporting solutions on the accuracy and appearance of the fat test. The curdy or charred material frequently en-

countered at the base of the fat column of Babcock tests on homogenized milk not only interferes with the accuracy of the test, but often gives to the test such an undesirable appearance as to cause the operator to have no confidence in its accuracy. The elimination of this foreign material through the making of accurate tests is an essential in a desired test. However, this is not always possible even with the best procedure. Consequently elimination of the plug of material completely from the fat column is next to be desired. To this end procedures recommended by Babcock (4), Hunziker (21) and

TABLE 6

The clarity of the Babcock fat column of homogenized milk fat tests as influenced by the addition of various mixtures in place of the last water added following centrifuging

Sample	Mojonnier fat test (%)		Babcock fat test (%)		Quintuplicates	Percentage fat and clarity of fat column of tests† on homogenized milk when fat column was supported by					
						Water		Hot dilute acid (1: 1)		Water-alcohol (1.4: 1)	
	NH*	H	NH*	H		%	Clarity	%	Clarity	%	Clarity
A	3.68	3.66	3.60	3.5	1	3.5	Cloudy; 1/16" black slug	3.4	Clear; 1/64" char	3.5	Clear; no sediment
					2	3.4	Sl. cloudy; feathery	3.5	Clear; 1/64" char	3.55	Clear; no sediment
					3	3.5	Clear; sl. feathery	3.45	Clear; 1/64" char	3.6	Clear; no sediment
					4	3.5	Cloudy; 1/8" char	3.5	Clear; 1/64" char	3.5	Clear; no sediment
					5	3.5	Cloudy; 1/32" char	3.5	Clear; 1/64" char	3.6	Clear; sl. feathery
Average						3.48		3.47		3.55	

* Key: NH indicates not homogenized; H indicates homogenized.
† Regular Babcock procedure followed.

Brueckner (7) were tried out. The results are presented in table 6. When hot dilute sulphuric acid was used instead of hot water, to bring the fat up into the neck of the bottle before final centrifuging, the fat column was unusually clear but black char was invariably present at the base of the fat column. On the other hand, substitution of a water-alcohol mixture for the final addition of water not only resulted in a clear fat column but one free from sediment. Any sediment present in the test was noticeable at the base of the water-alcohol column rather than at the base of the fat column.

Furthermore water-alcohol-supported fat columns averaged slightly higher in reading than did those supported by water or by dilute acid suggesting the possible solution of alcohol in the fat.

Temperatures attained in modified Babcock test. Final temperatures of the milk-acid mixtures in the modified Babcock tests were slightly lower than those of the regular Babcock test. This resulted wholly or in part from (a) the use of less acid; (b) the use of lower specific gravity acid; (c) the addition of the acid in small portions; or (d) prolonged shaking between additions of acid thus permitting partial cooling. Temperatures noted in one trial of test No. 8 involving 17 ml. of 1.835 sp. gr. acid added in three portions, 6, 6, and 5 ml., were 74°, 94°, and 101° C. (213.8° F.), respectively. The maximum temperatures of these modified Babcock tests are several degrees lower than the average maximum temperature of 219.2° F. observed in the regular Babcock test by Fahl, Lucas, and Baten (16). The influence on the final temperature of the acid-milk mixtures by variations in the amount or specific gravity of the acid may be noted in figures 1 and 2.

SUMMARY

Twelve modifications of the Babcock test for homogenized milk were studied to determine their accuracy and to ascertain which methods were most effective in eliminating the plugs of curdy or charred material at the base of the fat column.

Five series each of lower-fat and higher-fat homogenized milk were tested by each of ten modified methods as well as by the Mojonnier and by the regular Babcock method.

No one test was found consistently to eliminate completely the foreign material at the base of the fat column of tests on homogenized milk although some tests reduced the amount of this material to a negligible quantity.

The modified methods, yielding results both on lower-fat and on higher-fat homogenized milk closer to those of the Mojonnier and to those of the regular Babcock test on nonhomogenized milk, had in common (a) the use of the approximate or full amount of normal strength acid required of the Babcock test; (b) the addition of the acid in at least three portions; and (c) the remixing of the acid-serum-water mixture after centrifuging and following the addition of water.

Clear, char-free fat columns were obtained by the addition of a water-alcohol mixture (1.4 to 1) to the test as suggested by Brueckner (7) instead of the final addition of water.

Apparently in making a good Babcock fat test of homogenized milk, the reaction of the sulphuric acid on the proteins of the milk, particularly on that adsorbed to the fat globules, must be prolonged and be as vigorous as possible without engendering sufficient heat to char the sample.

Since this study was completed and the manuscript written, the paper

"The Babcock Fat Test on Homogenized Milk" by L. M. Lampert and J. H. Brandon has appeared in the Journal of Milk Technology **8**(3): 140–144, 1944, and hence has not been included in the review of literature.

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THE DETERMINATION OF TOTAL SOLIDS IN LIQUID DAIRY PRODUCTS BY DRYING WITH FORCED HEATED AIR. II. RESULTS WITH ICE-CREAM MIX AND SWEETENED-CONDENSED MILK*

F. J. DOAN AND C. W. LIVAK

The Pennsylvania Agricultural Experiment Station, Sate College, Pa.

In a previous report (1) the Dietert Moisture Teller was illustrated, described, and shown to be employable in the drying of a number of liquid dairy products for the purpose of accurately determining the total solids. This paper deals with its use in analyzing ice-cream mix and sweetened-condensed milk, the solids of which are difficult to dry due to the added sugar present and, in the case of ice cream, added colloidal stabilizers as well.

Official and Tentative Methods of Analysis of the A.O.A.C. (2) makes no mention of a method for determining the total solids of ice cream or ice-cream mix. There is, however, an "Official Method" for sweetened-condensed milk which has been recognized for many years.

PRELIMINARY STUDIES

Since there is no generally recognized standard method for ice cream which might serve as a standard of comparison for the development of a satisfactory Dietert procedure, it was decided to study a number of different modifications of the "Official Method" for sweetened-condensed milk and to select as a standard the one giving the lowest and most consistent results. To do this, eleven different samples of ice-cream mix were analyzed in triplicate for total solids by ten different modifications of the "Official Method" for sweetened-condensed milk. Eleven different samples of sweetened-condensed whole milk were also analyzed concurrently in identical manner.

Without going into tedious detail the modifications used compared: 1) dilution of the samples as called for in the "Official Method" with direct weighing and mixing with water in the dish; 2) the use of ignited sand to spread the sample with mere flowing of the sample over the bottom of the dish; 3) the use of a hot plate at 180° C. to dry the samples to a faint brown color with the use of a steam bath for 30 minutes, before final drying; 4) the utilization of an ordinary constant temperature oven with a vacuum oven at 100° C.; and 5) drying completely in an ordinary oven with pre-drying on a steam bath or hot plate followed by oven drying. In all cases the samples were dried to constant weight which required from two and one-half to six and one-half hours with the different modifications for ice-cream mix and

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from three to seven and one-half hours for sweetened-condensed milk under similar treatment.

Drying was hastened by the use of smaller samples, by pre-drying on a hot plate or steam bath and by the use of sand to create more surface but was not significantly accelerated by the use of a vacuum oven.

With sweetened-condensed milk it was found essential to dilute the sample and to use a material (sand) to increase the evaporative surface as called for in the "Official Method." All modifications of the method produced higher and more variable results. With ice-cream mix, three of the modifications produced results which averaged as low, or even a little lower than the "Official Method" for sweetened-condensed milk but they were definitely less consistent. The advantages of diluting the sample and of using sand were not as apparent with ice-cream mix as with sweetened-condensed milk. The modifications suggested by Fisher and co-workers (3, 4) were found to be quite satisfactory for ice-cream mix but not for sweetened-condensed milk. Five of the procedures used with ice-cream mix gave evidence of producing satisfactory results with further slight changes. However, it was felt that the variations encountered with ice-cream mix indicated the same trends as were evident in the data obtained with sweetened-condensed milk, although in less exaggerated form. Consequently, it seemed justifiable to conclude that the "Official Method" for sweetened-condensed milk represented the best procedure, of those studied, for determining the total solids content of ice-cream mix. This method, therefore, was used as a standard of comparison in the subsequent studies involving the Dietert Moisture Teller.

METHODS AND PROCEDURE

The methods used in selecting a satisfactory Dietert procedure for determining the total solids content of ice-cream mix and sweetened-condensed milk paralleled those explained in the previous paper (1).

As a result of the experience with plain-condensed milk and evaporated milk, wherein two-gram samples and temperatures under 120° C. gave unsatisfactory results with the Dietert apparatus, only one-gram samples and temperatures of 120° C. and over were utilized with ice-cream mix.

Six samples of ice-cream mix varying in composition from 8.66 to 13.15 per cent fat; 13.0 to 16.0 per cent sugar; and 31.14 to 38.65 per cent total solids (Mojonnier) were analyzed in triplicate using two different procedures for weighing out a sample, drying temperatures of 120° C. and 130° C. and drying intervals of 20, 25 and 30 minutes. In all, 216 individual analyses were made and the results were judged by comparison with analyses made in triplicate by the "Official Method" for sweetened-condensed milk, previously discussed.

Greater difficulty was anticipated, and realized, in selecting a satisfactory Dietert procedure for sweetened-condensed milk. It was found im-

possible to remove all the moisture from this product when the drying temperature was 130° C. without utilizing an excessive period of drying, even when samples equivalent to only four-tenths of a gram of product were used. Six samples of commercially manufactured sweetened-condensed whole milk were analyzed in triplicate using three procedures for weighing out a sample, drying temperatures of 140° C. and 150° C. and drying intervals of 20, 25, and 30 minutes. In all, 324 individual analyses were made and the results evaluated by comparison with the "Official Method."

After what appeared to be a satisfactory procedure for use with the Dietert Moisture Teller in analyzing ice-cream mix and sweetened-condensed milk had been established, a limited number of samples were analyzed in triplicate using the selected procedure and the results compared with the "Official Method" for the sake of confirmation. For comparative purposes, they were also analyzed in triplicate by the Mojonnier method (5).

All analyses were made using tared flat-bottomed aluminum dishes, 75 mm. in diameter.

RESULTS

Ice-cream mix. The procedure selected for determining total solids in ice-cream mix by means of the Dietert apparatus on the basis of previously mentioned trials is as follows:

A flat-bottom aluminum dish, 75 mm. in diameter, is heated in the Dietert apparatus for at least 10 minutes at approximately 130° C., during which time the temperature is carefully checked and adjusted. The dish is cooled in a desiccator to balance temperature and weighed with a suitable cover using an analytical balance. Approximately one gram of well-mixed sample is quickly weighed into the dish (weighing pipettes may be employed). One ml. of warm water is added and the dish contents well mixed and spread evenly over the dish. The dish is then placed in the center of the pan of the Dietert Moisture Teller in a level position. Forced-air drying should be continued for 30 minutes at 130° C., the temperature being checked again. After drying, the dish and residue should be immediately transferred to a desiccator, cooled, weighed and the weight of the dry solids determined.

The accuracy obtainable with this method is illustrated by the data in table 1 covering the analyses of five samples of ice-cream mix and presenting similar analyses made by the method selected as a standard and by the Mojonnier method.

The results indicate that the Dietert apparatus is capable of producing more accurate and more consistent results than are obtainable with the Mojonnier test as now conducted, judgment being based on the use of the "Official Method" for sweetened-condensed milk as a standard of comparison. The accuracy of the method is, therefore, deemed satisfactory.

The data cast some suspicion on the ability of the Mojonnier Tester to dry completely a sample of ice-cream mix, using the customary procedure

of drying a one-gram sample, mixed with one ml. of water, in the vacuum oven for ten minutes at 100° C. If the "Official Method" for sweetened-condensed milk is a satisfactory method for ice cream as has been indicated, then it would seem that ice-cream samples might require a longer drying period in the Mojonnier oven as has been found necessary with samples of sweetened-condensed milk.

TABLE 1

Accuracy of the selected Dietert procedure for determining total solids of ice-cream mix as judged by results obtained with the "Standard Method" and as compared with those found by the Mojonnier method

Per cent T. S. "Standard"	Results with Dietert apparatus		Results with Mojonnier tester	
	T. S. per cent	Variation from "Standard"	T. S. per cent	Variation from "Standard"
36.42	36.62	+0.20	36.89	+0.47
	36.50	+0.08	37.00	+0.58
	36.44	+0.02	36.96	+0.54
38.43	38.40	−0.03	38.65	+0.22
	38.40	−0.03	38.65	+0.22
	38.34	−0.09	38.59	+0.16
31.86	32.07	+0.21	32.15	+0.29
	31.99	+0.13	32.22	+0.36
	31.98	+0.12	32.17	+0.31
34.31	34.42	+0.11	34.63	+0.32
	34.38	+0.07	34.54	+0.23
	34.24	−0.07	34.41	+0.10
36.21	36.39	+0.18	36.58	+0.37
	36.25	+0.04	36.56	+0.35
	36.24	+0.03	36.44	+0.23
Algebraic average		+0.079		+0.317
Arithmetic average		±0.094		+0.317
Range		−0.09 to +0.21		+0.10 to +0.58

Sweetened-condensed milk. The procedure which seemed to give the most satisfactory results with sweetened-condensed milk when using the Dietert Moisture Teller for determining total solids was identical with that given previously for ice-cream mix except in the following particulars:

1. Two grams of a sample previously diluted one to two by weight with water are weighed into the solids dish or 2 ml. of a sample made up by weighing 33 grams into a 100-ml. volumetric flask and diluting to the mark with water, may be measured into the dish. An alternate procedure which gives somewhat less consistent results is to weigh directly into the dish somewhat less than one gram of sample and then mix with about 2 ml. of hot water.

2. The sample is dried in the Dietert apparatus for 20 minutes at a temperature of 150° C.

It was noted that in all trials using the Dietert apparatus, the sweetened-condensed milk residues, unless somewhat charred in appearance, gave high

total solids results. It appeared necessary to brown the solids definitely in order to remove sufficient moisture in a reasonably short time so that results would correspond to those obtained with the "Official Method."

In order to verify the accuracy of the selected Dietert procedure, five samples of commercial sweetened-condensed whole milk and two samples of sweetened-condensed skim milk were analyzed in triplicate, using two-

TABLE 2

Accuracy of the selected Dietert procedure for determining total solids of sweetened-condensed whole milk and skim milk as judged by results obtained with the "Official Method" and as compared with those found by the Mojonnier method

Per cent T. S. "Official"	Results with Dietert apparatus		Results with Mojonnier tester	
	T. S. per cent	Variation from "Official"	T. S. per cent	Variation from "Official"
Sweetened-condensed whole milk				
70.59	70.64	+0.05	70.90	+0.31
	70.83	+0.24	70.70	+0.11
	70.46	−0.13	71.29	+0.70
72.41	72.21	−0.20	73.10	+0.69
	72.79	+0.38	72.94	+0.53
	72.29	−0.12	73.08	+0.67
69.88	70.39	+0.51	70.16	+0.28
	70.15	+0.27	70.34	+0.46
	70.09	+0.21	70.07	+0.19
73.27	73.71	+0.44	73.13	−0.14
	73.74	+0.47	Lost	.....
	73.48	+0.21	73.74	+0.47
71.36	71.32	−0.04	72.18	+0.82
	71.50	+0.14	71.99	+0.63
	71.77	+0.41	71.97	+0.61
Sweetened-condensed skim milk				
69.63	70.03	+0.40	70.39	+0.76
	70.22	+0.59	69.84	+0.21
	69.70	+0.07	69.98	+0.35
71.22	71.91	+0.69	71.79	+0.57
	71.48	+0.26	71.13	−0.09
	71.76	+0.54	71.67	+0.45
Algebraic average		+0.280	.....	+0.440
Arithmetic average		±0.303	.....	±0.452
Range		−0.20 to +0.69	.....	−0.14 to +0.82

gram samples of the products diluted one to two by weight with water prior to weighing into the dishes. The same samples were also analyzed by the Mojonnier Method (5) using 90 minutes of vacuum oven drying. The results are shown in table 2 where they are compared with averages obtained from triplicate determinations performed by the "Official Method."

The results obtained with the Dietert Moisture Teller on sweetened-condensed milk are considered quite satisfactory inasmuch as this is undoubt-

edly the most difficult fluid dairy product to analyze for solids. The data indicate that the method gives somewhat closer agreeing results to the "Official Method" than does the Mojonnier method which in this study agreed somewhat better than was found to be the case by Fisher and Rice (3). It might be pointed out that somewhat more accurate results were obtained with the Mojonnier test when the samples were diluted previous to weighing in the case of two samples analyzed in this fashion. This might indicate that the water added to the dish after the sample is weighed-in does not always properly disperse the sample over the bottom of the dish.

In all of the work with sweetened-condensed milk it was found that the care with which the details of the procedures were carried out noticeably influenced the results. Triplicate checks as well as agreement with the "Official Method" improved with the experience of the operator. Undoubtedly the same conditions hold for the analysis of some of the other products, but because of the greater ease of checking results they are not so evident.

It should also probably be mentioned that when samples were weighed directly into the dishes and then diluted and dried with the Dietert apparatus, as is standard for the Mojonnier procedure, the results did not agree quite as well with the "Official Method" as did the Mojonnier analyses given in table 2.

CONCLUSIONS

The Dietert Moisture Teller, an apparatus employing forced heated air, is capable of drying ice-cream mix, sweetened-condensed whole milk and sweetened-condensed skim milk conveniently and reasonably accurately for the purpose of determining the total solid content. The results obtainable with the procedures given are more accurate than the commonly used Mojonnier techniques when the "Official Method" for sweetened-condensed milk is used as a standard of comparison.

The data shown raise a question as to whether the Mojonnier procedure as commonly used for ice-cream mix should not specify a longer drying period, as has been found necessary in the case of sweetened-condensed milk.

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THE VALUE OF THE ADDITION OF FRESH BAKERS' YEAST TO A NORMAL RATION FOR LACTATING DAIRY COWS

C. L. NORTON

Department of Animal Husbandry, Cornell University, Ithaca, N. Y.

INTRODUCTION

Numerous feeding trials have been conducted in this country and in Europe on the nutritional value of yeast. However, the results of experiments with yeast in the rations of domestic animals have not been consistent because of differences in the kind of yeast, the class of experimental animals, and feeding practices.

Bunger (3) found that the digestibility of the protein of wood sugar yeast was equal to that of crude extracted soybean oil meal; and that yeast and soybean oil meal were of equal value for milk production, per kilogram of crude protein consumed. Barton, Ness, and Crampton (1) concluded that dried brewers' yeast could be substituted for linseed meal pound for pound in the rations of dairy cows. Krauss and Hunt (5) fed dried yeast to dairy cows in amounts up to three-quarters of a pound per cow per day, and found no increase in the vitamin B content of the milk although there was some indication that riboflavin was slightly increased. Golf and Seeling (4) showed that feeding irradiated and non-irradiated yeast to dairy cows had no effect on the amount or fat content of the milk produced. Bilek and Hynek (2) obtained an increase in milk production with no change in the fat content when irradiated yeast was added to the winter ration of dairy cows, but when the cows were fed ample supplies of fresh fodder, the effect was no longer present.

Lucadou (6) fed 5 grams of living bakers' yeast to dairy cows which resulted in an increased fat test, but milk production remained unchanged. The same quantity of dried living brewers' yeast proved unpalatable and had no effect on either the amount of milk produced or the fat test. Feeding 50 mg. of vitamin B_1, or 6 mg. of vitamin B_2 separately failed to give positive results. However, when 20 mg. of B_1 and 6 mg. of B_2 were fed together, this investigator noticed a distinct rise in the fat content of the milk. Under winter feeding conditions, ten times as much vitamin B_1 and B_2 were required to give the response obtained with cows on pasture.

EXPERIMENTAL

This experiment was designed to test the reliability of the results reported on the beneficial effect of bakers' yeast in raising the fat test of milk. The object of the experiment was to determine whether or not the addition

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of fresh bakers' yeast to a normal ration would increase the fat test or the total fat yield of high-producing dairy cows.

Two groups of six cows each were used. With the exception of one cow in each group that was in her second lactation, all cows were two-year-olds in their first lactation periods. The cows were grouped according to the stage of lactation as well as the amount of fat and 4 per cent fat-corrected milk (F.C.M.) produced during a three-weeks' preliminary period. It was considered more advantageous to balance the two groups as to the stage of lactation than to have the groups alike in the amount of fat produced. It was impossible to equalize the two groups in respect to both factors. A

TABLE 1

Allotment of cows at the beginning of the experiment

Cow	Age	Stage of lactation	Average daily production	
			4% F.C.M.	Fat
	months	*days*	*pounds*	*pounds*
Yeast group				
Blend Lady	39	251	42.3	1.65
Pledge Louise	37	251	47.9	1.95
Pledge Tulip	33	168	56.7	2.25
Pledge Helena	30	79	62.3	2.29
Pledge Bessie	29	52	56.2	2.26
Hengerveld Sylvia	47	95	66.0	2.37
Average	35.8	149	55.2	2.13
Control group				
Pledge Edie	41	248	55.3	2.26
Ollie Pledge Rachel	35	177	51.6	2.10
Pledge Alfa	36	214	50.3	1.87
Successor Lydia	31	111	47.8	1.76
Pledge Gin	32	138	52.6	2.10
Lonsdale Sylvia	50	79	52.2	1.72
Average	37.5	161	51.6	1.97

two-weeks' standardization or preliminary period preceded the first experimental period when yeast was added to the ration.

The allotment of the experimental cows is shown in table 1.

The experimental cows were milked and fed grain three times daily. Yeast was fed each day at the noon feeding. Both groups of cows were fed the same rations with the exception that one group received yeast. The experimental cows received the same management as the regular milking herd. Four cows in each group were kept in individual box stalls, and each was fed a daily ration of 16.5 pounds of grain, 9 pounds of wet beet pulp, and mixed hay *ad libitum*. The other two cows of each group were stabled in tie stalls, and were fed a daily ration of 16.5 or 18 pounds of grain, 9 pounds of wet beet pulp, mixed hay *ad libitum* and 40 pounds of silage. Although the feeding varied among individuals in both groups, the effect of this was equalized because paired animals in each group received

the same treatment. At the beginning of the eighth week of the experiment when the yeast feeding was increased to 80 grams a day, the cows were turned out to pasture. The rate of grain feeding was reduced three pounds per day at this time.

The 12-week experimental period was divided into three 4-week periods. Fresh bakers' yeast was fed to one group at the rate of 20 grams per cow per day for the first four weeks. During the second period, the amount of yeast fed was doubled. In the final period, yeast was fed at the rate of 80 grams per cow per day. This is shown in figure 1.

The yeast was fed in the following manner: The amount of yeast to be fed for one day to the six cows receiving yeast was weighed, and then placed

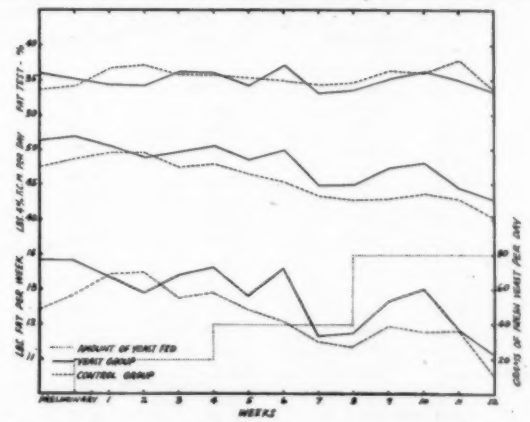

FIG. 1. Trends in fat test, production of 4% fat-corrected milk, and production of total fat.

in a water suspension by mixing thoroughly in a Waring blender for about two minutes. The total amount was divided into six equal portions and was poured directly on the grain.

The bakers' yeast was supplied for the experiment by the Utica, New York, Branch of Anheuser-Busch, Incorporated. It was shipped weekly and was stored at 35° F. until used.

Analysis of the fresh compressed bakers' yeast which contained 68.3 per cent moisture showed it contained the following vitamins per gram of fresh yeast: thiamin, 7.0 μg.; riboflavin, 16.5 μg.; pantothenic acid, 21.8 μg.; nicotinic acid, 141.8 μg.; pyridoxine, 35.6 μg.; folic acid, 0.9 μg.; and biotin, 0.02 μg.

DISCUSSION

The production of the cows in both groups was exceptionally high. Fat test, fat production, and milk production figures for the yeast-fed and the

control groups are given in tables 2 and 3. The average fat test was greater in the yeast-fed group at the beginning of the trial, but at the end of the experiment the reverse was true. In total fat production, the yeast-fed group was higher than the control group throughout most of the experiment although the difference between the groups was smaller at the end of the experimental period.

At the beginning of the experiment, the production of 4 per cent F.C.M. in the yeast-fed group averaged 51.9 pounds per day compared with 48.3 pounds per day for the control group. In the yeast-fed group, milk pro-

TABLE 2

Production summary of group fed fresh bakers' yeast

Week	Daily milk	Fat test	Daily fat	Daily 4% F.C.M.
	pounds	*per cent*	*pounds*	*pounds*
Preliminary	54.9	3.61	1.98	51.7
Preliminary	56.0	3.54	1.98	52.1
Average	55.5	3.57	1.98	51.9
1	55.3	3.45	1.91	50.7
2	53.7	3.45	1.85	49.2
3	52.7	3.64	1.92	49.9
4	54.0	3.63	1.96	50.9
Average 1–4 weeks	53.9	3.54	1.91	50.2
5	53.4	3.45	1.84	48.9
6	52.4	3.72	1.95	50.3
7	50.1	3.33	1.67	45.1
8	50.1	3.37	1.69	45.3
Average 5–8 weeks	51.5	3.47	1.79	47.4
9	51.3	3.55	1.82	47.8
10	51.3	3.65	1.87	48.5
11	48.4	3.53	1.71	44.9
12	48.0	3.33	1.60	43.2
Average 9–12 weeks	49.8	3.51	1.75	46.1
12-weeks average	51.7	3.50	1.81	47.9

duction dropped 16.7 per cent to 43.2 pounds, an average decline per week of 1.39 per cent. In the control group, production was maintained only slightly better. It dropped from 48.3 pounds per day to 40.7 pounds which was 15.7 per cent over the 12-week experimental period, an average decrease in production of 1.31 per cent per week. In other words, the persistency of the two groups of cows was essentially the same. These trends are shown in figure 1.

When fed at the rate of 80 grams per day, the yeast seemed to be unpalatable to some of the cows. Whether this was due to the taste of the yeast or to the change in physical characteristic of the feed when moistened was not determined. When the yeast was fed on the wet beet pulp, the cows had no objection to it. The yeast was not so unpalatable in any case that feed was refused.

The health of the cows during the experiment was relatively good. One cow in the yeast-fed group, Helena, was off feed during the sixth week for some unknown reason. Beginning the ninth week, four cows, Lydia, Alfa, Tulip, and Rachel, became infected with cowpox, but it was of short duration and did not affect production. No other health disturbances were observed.

Since the amount of nutrients in the yeast that was added to the ration of the yeast-fed group was so small as to be covered up by inaccuracies in

TABLE 3

Production summary of control group

Week	Daily milk	Fat test	Daily fat	Daily 4% F.C.M.
	pounds	*per cent*	*pounds*	*pounds*
Preliminary	52.6	3.38	1.78	47.7
Preliminary	53.5	3.42	1.83	48.9
Average	53.1	3.41	1.81	48.3
1	52.2	3.70	1.93	49.8
2	51.8	3.73	1.93	49.7
3	50.9	3.60	1.83	47.8
4	51.6	3.59	1.85	48.4
Average 1–4 weeks	51.6	3.66	1.89	48.9
5	50.3	3.56	1.79	46.9
6	49.5	3.52	1.74	45.9
7	47.6	3.47	1.65	43.8
8	46.7	3.49	1.63	43.2
Average 5–8 weeks	48.5	3.51	1.70	45.0
9	46.9	3.67	1.72	44.5
10	46.8	3.63	1.70	44.2
11	44.6	3.81	1.70	43.3
12	44.9	3.39	1.52	40.7
Average 9–12 weeks	45.8	3.62	1.66	43.2
12-weeks average	48.6	3.60	1.75	45.7

the weighing of feed, waste in eating, and variation in the amount of hay consumed, no compensating adjustment in the ration of the control group was made.

CONCLUSIONS

With the type of fresh bakers' yeast used in this experiment fed in increasing daily amounts from 20 grams to 80 grams per cow, it was impossible to show any significant difference in the fat test, the amount of fat produced, or the amount of 4 per cent F.C.M. yielded over a 12-week experimental period.

Feeding living bakers' yeast up to 80 grams per cow per day had no apparent beneficial effects on the appetites of the cows. No injurious effects of this level of yeast feeding on the health of the cows were observed.

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CHANGES IN THE LEVEL OF VITAMIN A AND CAROTENE IN THE BLOOD PLASMA OF DAIRY COWS ASSOCIATED WITH PARTURITION AND BEGINNING LACTATION

T. S. SUTTON, H. E. KAESER, AND P. A. SOLDNER

The Ohio Agricultural Experiment Station and The Ohio State University

In a study of the normal levels of vitamin A and carotene in the blood plasma of dairy cows (10) it soon became apparent that the level of these substances in the blood changed markedly at the time of parturition and beginning lactation. A search of the literature at that time revealed one isolated report in substantiation of this observation. Gallup and Kuhlman in 1941 (5) observed a decrease in carotene in the blood plasma of 13 out of 19 cows.

Since the date of our observation of this change, Sutton and Soldner (9), Kuhlman and Gallup (7), and Braun (3) have reported on this subject. Sutton and Soldner reported a decrease in both blood-plasma carotene and vitamin A. Kuhlman and Gallup reported carotene decreases averaging as high as 30 per cent in cows having blood-plasma carotene levels above 200 micrograms per ml. previous to parturition. They did not study possible vitamin A changes. Braun reported a decrease in blood-plasma vitamin A at the time of parturition which was independent of fluctuations in the carotenoid level.

In view of the paucity of data on this subject, an experiment designed more carefully to investigate this matter was considered advisable.

EXPERIMENTAL

Experimental animals. Twenty-eight cows in the Ohio State University dairy herd were used in this experiment. These cows consisted of 7 each of the Ayrshire, Jersey, Guernsey, and Holstein breeds. These cows all freshened during the winter months while they were on a barn feeding schedule. They were housed with the rest of the herd and fed a good quality mixed alfalfa hay, corn silage, and a concentrate mixture. No special effort was made to treat these cows as experimental animals. Carotene intake was not determined, although blood-plasma carotene and vitamin A levels of these cows at the beginning of the test period, and of other individuals in the herd at the same time, indicated adequate carotene intakes.

Sampling procedure. In planning the experiment it was decided to obtain blood samples for analyses at weekly intervals beginning 4 weeks prior to the expected parturition date and continuing to 2 weeks post-partum. Since the parturition date was considered the critical date in the schedule,

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other samples were to be obtained within 24 hours previous to and following parturition and 3 days following parturition. The latter bleeding was decided on because it would come close to the end of the colostrum-production period.

Since the exact prediction of the date of parturition is quite inaccurate, obtaining blood samples on the exact weekly interval previous to parturition is impossible. The majority of the cows in this experiment calved in advance of the calculated parturition date; therefore, the data for those samples which were actually drawn 4 weeks or more pre-partum are not included in the presentation of the data in table 1. The remaining data were allotted to the weekly interval falling nearest to the actual date of bleeding. No 3-week-prefreshening sample was obtained from one cow and no prefreshening samples were obtained from 7 cows. With these exceptions all samples were obtained and assayed as scheduled.

Colostrum samples. Inasmuch as colostrum is known (4, 8, and others) to be sufficiently high in carotene and vitamin A to constitute a severe drain on the body reserves, a quantitative determination of the output for the first 3 days post-partum was made. The calves were not allowed to nurse but were hand-fed from nipple pails from birth. The colostrum was hand-milked and weighed at each milking. A composite sample was obtained from each day's production and assayed for vitamin A and carotene. In two instances when parturition occurred during the night and there was evidence that the calf had nursed when first observed, no samples were taken. Therefore, colostrum-output data presented herein were obtained from only 6 of the Guernseys and 6 of the Jerseys in the experiment.

Assay procedure. Vitamin A and carotene were extracted from the blood plasma according to the method of Kimble (6). When the carotene concentration was less than 300 micrograms per 100 ml. of plasma, the vitamin A determination was carried out by Kimble's (6) method. The vitamin A of samples with carotene concentration exceeding 300 micrograms per 100 ml. was determined by the method of Boyer, Phillips and Smith (1).

The vitamin A and total carotenoids of the colostrum was determined by the method of Boyer *et al.* (2). Because of the tremendous concentration of vitamin A and carotene in colostrum in comparison with normal milk, an adaptation of the method to a smaller sample was made. This constituted the only modification.

The concentration of both vitamin A and carotenoid was measured with an Evelyn Photoelectric Colorimeter using the appropriate filters. The instrument was previously standardized against crystalline vitamin A alcohol in petroleum ether solution and crystalline beta carotene in chloroform solution.

RESULTS

The changes in blood-plasma vitamin A and carotene during the 5-week

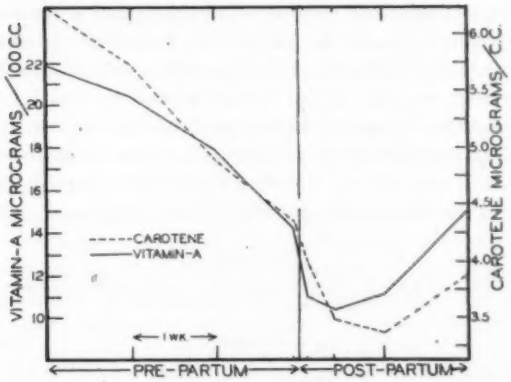

FIG. 1. Changes in blood-plasma carotene and vitamin A associated with parturition.

period of investigation are presented in table 1 and figure 1. Average data for each of the four breeds studied are presented in table 1. Figure 1 presents the data for the entire group of 28 cows.

The data on vitamin A and carotene output in the colostrum are summarized in table 2. Tabular presentation of individual animal data, both in this instance and in case of the blood plasma, is too voluminous for publication here. They are available for reference, however, should the reader have a question concerning them.

DISCUSSION

The data presented in table 1 and figure 1 show some marked changes in the blood-plasma carotene and vitamin A at the time of parturition and of beginning lactation in dairy cows. As pointed out earlier in this paper, the

TABLE 1

Changes in blood-plasma carotene and vitamin A at parturition and beginning lactation. (Average of 7 cows of each breed)

Breed	Prefreshening data				Postfreshening data			
	3 wk.	2 wk.	1 wk.	12 hr.	12 hr.	3 da.	1 wk.	2 wk.
Blood-plasma vitamin A, micrograms/100 ml.								
Ayrshire	20.3	18.0	16.1	13.8	10.8	10.8	10.4	12.4
Guernsey	21.7	21.1	18.7	14.2	11.4	9.8	11.9	16.2
Holstein	23.1	21.7	18.2	14.4	10.7	10.2	10.2	15.8
Jersey	21.9	20.3	17.9	14.1	11.2	10.4	11.1	14.9
Blood-plasma carotene, micrograms/100 ml.								
Ayrshire	454	394	326	299	274	248	231	258
Guernsey	1009	822	769	628	580	534	548	668
Holstein	494	451	373	312	303	274	219	238
Jersey	612	594	514	463	473	357	373	425

cows used in this experiment were on winter feed and a gradual decrease in blood-plasma carotene could be expected. Therefore, in considering the blood-plasma carotene changes, it is impossible to ascertain how much of the decrease noted was due to the decline known to result from decreased carotene intake and how much was due to physiological changes during this period in the reproductive cycle. The rise in blood-plasma carotene following parturition is convincing evidence that there is a decrease in blood-plasma carotene associated with parturition and beginning lactation.

TABLE 2

Average potency and total output of vitamin A and carotene in the colostrum of four breeds of dairy cows

Breed	Cows	Vitamin A		Carotene	
		Potency	Total output	Potency	Total output
First day					
	No.	*γ/ lb.*	*γ*	*γ/ lb.*	*γ*
Ayrshire	7	1,386	30,656	1,325	29,497
Guernsey	6	1,048	18,228	2,347	41,079
Holstein	7	1,113	37,098	928	31,987
Jersey	6	1,029	17,434	1,097	15,770
Second day					
Ayrshire	7	588	13,852	522	12,532
Guernsey	6	424	11,507	905	23,876
Holstein	7	392	15,699	331	13,393
Jersey	6	633	14,167	646	13,011
Third day					
Ayrshire	7	390	9,544	346	8,893
Guernsey	6	200	6,123	484	14,650
Holstein	7	204	9,535	186	10,310
Jersey	6	400	11,552	401	11,115
Total 3-day output					
Ayrshire	7	.....	54,052	.....	50,922
Guernsey	6	.....	35,858	.....	79,605
Holstein	7	.....	62,332	.....	55,690
Jersey	6	.....	43,153	.....	39,896

Considering all animals together the low point in blood-plasma carotene was reached one week following parturition. The overall decrease amounted to 46 per cent of the 3-week pre-partum level. All animals showed a decrease; however, there was considerable individual variation both in the time following parturition at which the maximum decrease was reached and the magnitude of the decrease. Carotene reached the lowest level in the blood plasma of 13 of the cows 3 days following parturition; of 11, one week following; of 2, 2 weeks following; and of 1, within 24 hours following parturition. Individual variations in the magnitude of the decrease ranged from

a low of 35 per cent to a high of 69 per cent. When all the cows are considered as a group, the decrease in blood-plasma carotene amounted to 46 per cent of the 3-week pre-partum level. This is a substantially greater decrease than that reported by Kuhlman and Gallup (7), but, as previously stated, it is not known how much of the change can be charged to decreased carotene intake and how much is associated with the physiological changes of the period in the reproductive cycle.

The decrease in blood-plasma vitamin A began about 3 weeks pre-partum. This cannot be ascertained from the data in table 1; however, a 4 weeks' pre-partum blood sample was obtained from 13 cows in the experiment. The data from these samples, presented in table 3, demonstrate this point.

TABLE 3

Changes in blood-plasma carotene and vitamin A at parturition and beginning lactation of the 13 cows from which 4-week-pre-partum samples were obtained. (Results in micrograms per 100 ml.)

	Pre-partum data					Post-partum data			
	4 wk.	3 wk.	2 wk.	1 wk.	12 hr.	12 hr.	3 da.	1 wk.	2 wk.
Carotene	709	611	569	487	435	412	353	327	364
Vitamin A	21.6	22.3	19.7	17.6	16.0	11.6	10.5	10.8	13.9

As in the case of the carotene, a decrease was noted in every animal, and based on data reported earlier (10) for animals during the same period of the year, the major part of the decrease in blood-plasma vitamin A is associated with parturition and beginning lactation. There was considerable individual variation both in the time at which the maximum decrease in blood-plasma vitamin A was noted and the magnitude of the decrease. The lowest level found following parturition was 3.5 micrograms per 100 ml. of plasma in a Holstein cow that had a vitamin A level of 27.8 micrograms per 100 ml. of plasma 3 weeks pre-partum.

The lowest level of blood-plasma vitamin A was found in 12 of the cows 3 days following parturition; in 10, one week post-partum; in 5, within 24 hours; and in 1, 2 weeks post-partum. The low point in both vitamin A and carotene coincided in 12 of the 28 cows. Six of the 7 Guernsey cows are included in this group of 13. This may be merely a coincidence, but it gives rise to some speculation since it occurred in the breed with the greatest colostrum output of carotene.

The individual variation in percentage decrease of blood-plasma vitamin A was greater than for carotene. The decrease ranged from a low of 36 per cent to a high of 87 per cent. When all of the cows are considered together as a group the decrease in blood-plasma vitamin A was 52 per cent of the 3-week pre-partum level. It will be noted also in figure one that the low point in vitamin A occurred on the third day post-partum; while the low point in carotenoid was 1 week post-partum.

Data on the concentration of vitamin A and carotene in the colostrum and the total output in the first 3 days of lactation are summarized in table 2. It will be noted that the total output is sufficient to constitute a severe drain on the body reserves. Since there were large variations in the output as well as in the changes in blood levels, correlations between total output and percentage decrease in blood level were calculated. No statistical evidence of correlation was found. This was not particularly surprising when some of our observations were considered. There is little doubt that the secretion of colostrum drains carotene and vitamin A from the circulating blood. It was observed that associated with the rapid filling of the udder was a sharp decline in the blood-plasma level of carotene and vitamin A. This is well illustrated in figure 1 where the sharpest decline in these blood constituents occurred at the time of parturition. In most of the cows this is also the time at which the udder is most rapidly distended with secretion.

The magnitude of the decrease in blood-plasma carotene and vitamin A seems to depend in part at least upon the rate of secretion rather than on the total output during the 3-day colostrum-producing period. In those animals in which there was considerable congestion of the udder the percentage decrease in the blood plasma was less than in those with little udder congestion. In the former animals the potency of the colostrum did not fall so rapidly from the first to the second and third day. On the other hand, in the case of those animals which milked out well from the start the potency of the colostrum fell off sharply from the first to the second day and closely approached that of normal milk by the third day.

Braun (3) has reported a decrease in blood-plasma vitamin A associated with abortion and acute infection. This would indicate other physiological relationships which complicate any direct correlation between total output following normal parturition and blood-plasma level. It appears that the complexity of physiological factors involved does not permit a study of relationships between isolated factors such as total output and blood plasma levels.

The low level to which blood-plasma vitamin A falls at parturition and beginning lactation emphasizes the need for maintaining an adequate intake of carotene to insure sufficient body reserves for this period.

SUMMARY

The changes in blood-plasma vitamin A and carotene associated with parturition and beginning lactation were studied in 28 dairy cows. The total output of vitamin A and carotene in the colostrum during the first 3 days of lactation in 26 of these cows was also determined.

Both vitamin A and carotene were found to decrease markedly in the blood plasma at the time of parturition and beginning lactation. Under the conditions of this experiment the maximum decrease in blood-plasma caro-

tene was reached one week following parturition and amounted to 46 per cent of the 3-week pre-partum level.

The maximum decrease in blood-plasma vitamin A for the entire group was reached 3 days following parturition and amounted to 52 per cent of the pre-partum level.

The total output of vitamin A and carotene in the colostrum during the first 3 days post-partum averaged 48,847 micrograms of vitamin A and 56,524 micrograms of carotene.

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SOME EFFECTS OF THYROPROTEIN ON THE COMPOSITION OF MILK*

J. G. ARCHIBALD
Massachusetts Agricultural Experiment Station

Several investigators have demonstrated the effect of thyroprotein (synthetic thyroxine) on milk production in dairy cattle, but beyond noting frequent phenomenal increases in fat content and slight changes in solids-not-fat, have done little on the effect this hormone may have on the composition of milk. Jack and Bechdel (1) were unable to detect any influence of thyroxine on the composition of the milk from two cows; Jones (2) noted an average increase of 9 per cent in lactose content of the milk from eight cows; Smith and Dastur (4) using four cows found "no consistent change in the level of non-fatty solids in the milk except for a slight decrease in protein concentration." In all the above cases natural thyroxine was fed; VanLandingham *et al.* (5) fed thyroprotein to six cows and noted a 33 per cent decrease in ascorbic acid content of the milk.

EXPERIMENTAL

During the past winter (1944–45) further study of the subject was conducted at this station. Eighteen pure-bred cows (six each of the Ayrshire, Holstein, and Jersey breeds) were fed 15 grams of thyroprotein daily[1] for periods of six weeks. The feeding and management was that usually followed in this herd, the rations consisting of mixed hay, corn silage, grass silage, beet pulp, and an 18 per cent grain ration. The cows were kept in their accustomed places in the herd line-up and were machine-milked twice daily. Each day's portion of thyroprotein was mixed with the morning grain allowance. Pertinent data regarding the individual animals are given in table 1.

Individual milk samples were obtained from all cows in the trial just before the start and twice during its course. Determinations made on all samples were: total solids, fat, ash, total protein, and casein. Lactose, lactalbumin, and globulin were ascertained by differences. Total solids, fat, and ash were determined by the usual routine methods; total protein was determined by the Kjeldahl procedure (factor for nitrogen conversion to protein—6.39); casein was determined by the method of VanSlyke and Bosworth as described by Richmond (3). Results of the study are summarized

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[1] The dosage was halved for the three Jerseys used in the latter part of the trial, as the other three had lost considerable weight and had become very nervous toward the end.

in table 2, and are presented graphically in figures 1 to 4. Because the two groups of cows reacted so differently to the thyroprotein, it has been deemed best to present the results for the two halves of the trial separately; average figures would obscure almost completely some points brought out by the work.

DISCUSSION OF RESULTS

The tabulated results and the graphs tell the story without much further elaboration. Total solids, ash, and lactose changed so little that graphs for them are not warranted. The most marked changes were in the content of

TABLE 1

Status of the individual cows at the beginning of the trial

Cow No.	Age (yrs. and mos.)	Stage in lactation (days)	Milk yield (lbs. daily)	Stage of gestation (month)
1st half of trial—Nov. 28th to Jan. 9th				
A278	8–11	317	18	6
A309	6–2	246	16	6
A330	4–11	193	23	5
H407	3–8	77	51	2
H420	3–2	244	26	5
H570	5–2	249	25	6
J887	5–9	205	12	5
J910	3–11	411	14	6
J918	3–5	271	18	6
2nd half of trial—Feb. 10th to Mar. 24th				
A327	5–2	144	35	3
A341	4–4	158	23	4
A364	3–3	150	14	Open
H509	8–3	133	53	2
H567	5–5	165	46	3
H584	4–10	154	42	4
J852	8–6	136	22	1
J857	8–1	176	21	Open
J907	4–2	159	29	2

casein and albumin. In the first half of the trial the casein was lowered in the milk of all individuals, the amount of decrease ranging from a little over 1 per cent to 33 per cent. Also without exception the casein values dropped lower as this half of the trial proceeded. In the second half the decrease in casein values was not as large nor as consistent but it occurred in the majority of cases. Albumin increased roughly in proportion as casein decreased. Contrary to what might be expected in view of the published results of other investigators, fat percentage increased in only about half of the cases; in the second half of the trial the tendency was definitely for it to decrease. The rather striking behavior of the Jersey cows in this respect in the later group (see figure 1, lower section) possibly may be due to the fact that the

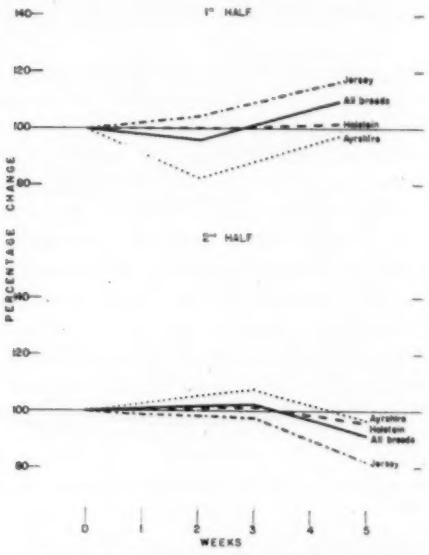

FIG. 1. Changes in fat content of milk.

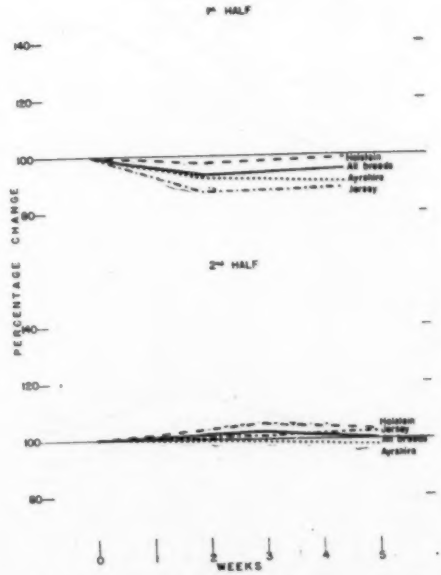

FIG. 2. Changes in total protein content of milk.

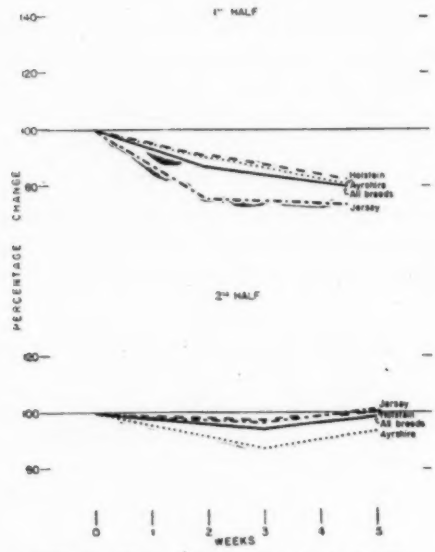

FIG. 3. Changes in casein content of milk.

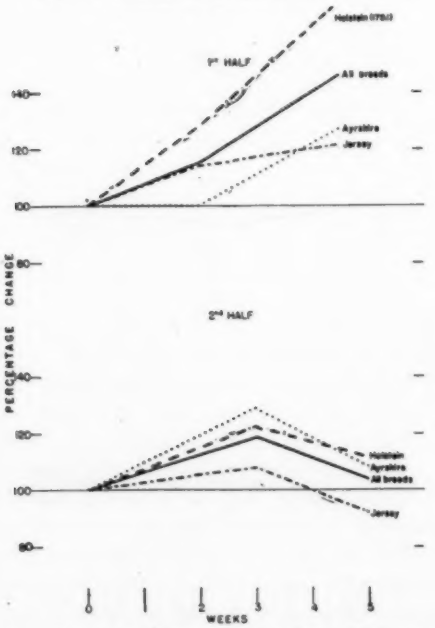

FIG. 4. Changes in albumin and globulin content of milk.

dosage of thyroprotein was halved for them for reasons given in the footnote on page 941.

With two exceptions variations between individuals within a breed were greater than variations between breeds; these exceptions were variations in albumin in Jersey milk, and variations in casein in Holstein milk. This matter of variation in response of individuals to thyroprotein apparently is of considerable consequence; in this work it has been noted not only in varying changes in milk composition but also in widely differing response in milk yield. Figure 5 shows the extremes in this respect as well as the breed averages. The decrease in fat content of the milk of the three Jerseys used in the second half of the trial (see the previous paragraph) was accompanied by a relatively low increase in milk yield—less than one-sixth as great on the average as that noted in the three Jerseys used in the first half (4.8 per cent in contrast with 29.4 per cent). Here again halving of the dosage of thyroprotein undoubtedly had somewhat to do with the result,

TABLE 2

Changes in composition of milk as a result of feeding thyroprotein

Breed and time	Total solids	Fat	Ash	Total protein	Casein	Albumin* and globulin	Lactose†
(a) 1st half of the trial							
	%	%	%	%	%	%	%
Ayrshires							
At start	13.93	4.79	0.71	3.73	2.92	0.81	4.70
After 2 weeks	13.02	3.97	0.74	3.46	2.65	0.81	4.85
After a month	13.22	4.65	0.74	3.38	2.35	1.03	4.45
Relative changes—‡							
At end of 2 weeks	−6.5	−17.1	+4.2	− 7.2	− 9.2	0.0	+3.2
At end of a month	−5.1	− 2.9	+4.2	− 9.4	−19.5	+27.2	−5.3
Holsteins							
At start	13.23	3.98	0.71	3.47	2.80	0.67	5.07
After 2 weeks	13.31	3.98	0.76	3.39	2.54	0.85	5.18
After a month	13.28	4.05	0.72	3.44	2.30	1.14	5.07
Relative changes—							
At end of 2 weeks	+0.6	0.0	+7.0	− 2.3	− 9.3	+27.9	+2.2
At end of a month	+0.4	+ 1.8	+1.4	− 0.9	−17.9	+70.1	0.0
Jerseys							
At start	16.40	6.10	0.77	4.53	3.12	1.41	5.00
After 2 weeks	16.12	6.37	0.79	3.97	2.36	1.61	4.99
After a month	17.17	7.41	0.77	4.01	2.30	1.71	4.98
Relative changes—							
At end of 2 weeks	−1.7	+ 4.4	+2.6	−12.4	−24.4	+14.2	−0.2
At end of a month	+4.7	+16.3	0.0	−11.5	−26.3	+21.3	−0.4
All breeds							
At start	14.10	4.68	0.72	3.77	2.90	0.87	4.93
After 2 weeks	13.85	4.51	0.76	3.54	2.54	1.00	5.04
After a month	14.33	5.11	0.74	3.58	2.31	1.27	4.90
Relative changes—							
At end of 2 weeks	−1.8	− 3.6	+5.6	− 6.1	−12.4	+14.9	+2.2
At end of a month	+1.6	+ 9.2	+2.8	− 5.0	−20.3	+46.0	−0.6

TABLE 2 (*Continued*)

Breed and time	Total solids	Fat	Ash	Total protein	Casein	Albumin* and globulin	Lactose†
			(b) 2nd half of the trial				
	%	%	%	%	%	%	%
Ayrshires							
At start	13.41	4.45	0.71	3.31	2.34	0.97	4.94
After 3 weeks	13.47	4.81	0.70	3.30	2.05	1.25	4.66
After 5 weeks	13.11	4.32	0.72	3.24	2.19	1.05	4.83
Relative changes—							
At end of 3 weeks	+0.4	+ 8.1	−1.4	− 0.3	−12.4	+28.9	−5.7
At end of 5 weeks	−2.2	− 2.9	+1.4	− 2.1	− 6.4	+ 8.2	−2.2
Holsteins							
At start	12.77	4.11	0.68	3.02	2.04	0.98	4.96
After 3 weeks	12.81	4.17	0.68	3.19	1.99	1.20	4.77
After 5 weeks	12.63	3.95	0.68	3.13	2.04	1.09	4.87
Relative changes—							
At end of 3 weeks	+0.3	+ 1.5	0.0	+ 5.6	− 2.5	+22.4	−3.8
At end of 5 weeks	+1.1	− 3.9	0.0	+ 3.6	0.0	+11.2	−1.8
Jerseys							
At start	16.14	6.63	0.75	3.75	2.37	1.38	5.01
After 3 weeks	15.60	6.49	0.75	3.79	2.30	1.49	4.57
After 5 weeks	14.54	5.33	0.73	3.65	2.38	1.27	4.83
Relative changes—							
At end of 3 weeks	−3.3	− 2.1	0.0	+ 1.1	− 3.0	+ 8.0	−8.8
At end of 5 weeks	−9.9	−17.9	−2.7	+ 2.7	+ 0.4	− 8.0	−3.6
All breeds							
At start	13.78	4.83	0.70	3.28	2.20	1.08	4.97
After 3 weeks	13.68	4.91	0.71	3.37	2.08	1.29	4.69
After 5 weeks	13.25	4.41	0.71	3.29	2.17	1.12	4.84
Relative changes—							
At end of 3 weeks	−0.7	+ 1.7	+1.4	+ 2.7	− 5.5	+19.4	−5.6
At end of 5 weeks	−3.8	− 8.7	+1.4	+ 0.3	− 1.4	+ 3.7	−2.6

* Total protein—casein.

† Total solids—[fat + protein + (ash × 0.7)].

‡ These are percentage changes; a value of 100 is assumed for the initial figure in every case. This has been done in order that changes for all constituents may be strictly comparable; also to enable plotting of all graphs on the same scale.

but it is unusual to see relatively low production accompanied by lower fat content of milk.

Losses in body weight were noted as the trial progressed, being somewhat greater in the first group than in the second. It was noted, however, that these losses could be fully restored by increasing the grain allowance in proportion to the increase in milk yield.

SUMMARY

Eighteen cows were used in a study of the effect of thyroprotein on milk composition. They were fed 15 grams daily of the hormone for periods of six weeks. The milk was sampled at the start and twice during the feeding period. Determinations made were: total solids, fat, ash, total protein.

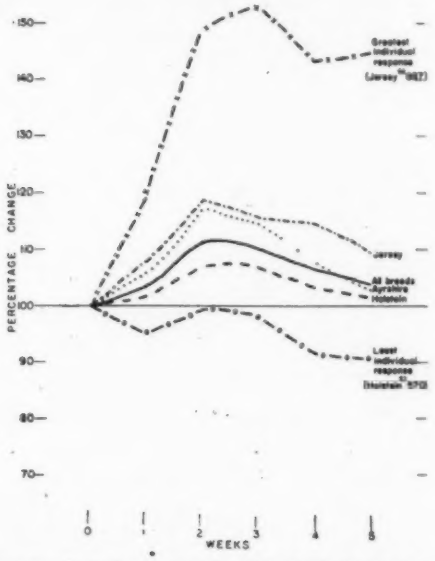

FIG. 5. Average trends and extremes in milk yield changes as a result of feeding thyroprotein.

and casein. Lactalbumin and globulin, and lactose were determined by difference.

Most important changes noted here were a considerable and rather consistent decrease in casein, and a roughly proportional increase in lactalbumin and globulin. Changes in fat content of the milk were not consistent, an increase being noted in only about half of the cases.

Variations in milk composition between individuals within a breed in the great majority of cases were greater than variations between breeds. Wide variations in milk yield response to the hormone were also noted.

These results should be considered as preliminary. It is planned to repeat the trials during the coming winter in a more intensive manner.

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CHEMISTRY

385. Spectrophotometric Determination of Calcium. ROBERT E. SCOTT AND C. R. JOHNSON, Univ. of Texas, Austin, Texas. Jour. Indus. Engin. Chem., Analyt. Ed., *17*, No. 8: 504–506. August, 1945.

A rapid and reasonably precise spectrophotometric method applicable to the determination of small and moderate quantities of calcium was developed. Calcium was precipitated as oxalate and determined by several variations as permanganate. A calibration curve adapts the method to the particular instrument being used, a spectrophotometer or a photoelectric colorimeter. Comparisons of results with official and standard methods show good agreement in determination of calcium in waters, brines, chemical reagents and food concentrates including dry whole milk. B.H.W.

386. The Amino Acid Composition of Human Milk Protein. MARTIN B. WILLIAMSON, Res. Lab. S.M.A. Corp., Chagrin Falls, Ohio. Jour. Biol. Chem., *156*, No. 1: 47–52. Nov., 1944.

It has been assumed that human milk for infant feeding is superior nutritionally to cow's milk. A better understanding of the amino acid composition of human milk as compared with cow's milk motivated this study.

For the analyses a five-gallon composite sample, representing approximately 500 collections from both white and Negro women, was used.

1. The casein and lactalbumin from human and cow's milk were isolated and the amino acid content for eighteen amino acids determined.

2. In a comparison of human milk and cow's milk diluted to 2 volumes, the analyses indicate that there is over 3 times as much cystine in human milk. However, less methionine was found in the human milk. On the basis of millimoles of total sulfur amino acids, there is no significant difference between the two.

3. The diluted cow's milk was shown to contain higher percentages of valine, threonine, and histidine while human milk was found richer in tryptophane.

Thirty references are given in the bibliography. A.O.C.

387. A Simplified Method for the Determination of Iron in Milk. W. R. RUEGAMER, L. MICHAUD, AND C. A. ELVEHJEM, Dept. of Biochem., Univ. of Wis., Madison, Wis. Jour. Biochem., *158:* 573–579. May, 1945.

A modification of the bipyridine method which requires no ashing is proposed for the determination of iron in milk. Iron values obtained by this

procedure range from 0.490 to 0.570 mg. per kilo of raw or market milk. For evaporated milk the range was from 1.144 to 1.234 mgs. per kilo.

A.O.C.

388. The Lysine Content of Casein and Zein. A. NEUBERGER, Nat'l. Inst. Med. Res., Hampstead, London. Jour. Biol. Chem., *158:* 717. May, 1945.

By the decarboxylase method casein was found to contain from 7.5 to 8.0 per cent lysine. Zein (the protein found in corn), on the other hand, contains only a negligible amount of lysine. A.O.C.

FOOD VALUE OF DAIRY PRODUCTS

389. Ascorbic Acid in Evaporated Milk. D. V. JOSEPHSON AND F. J. DOAN. Penn. Agr. Expt. Sta. Bul. 473. 22 pages. April, 1945.

The bulletin summarizes a four-year study of ascorbic acid in evaporated milk. Nutritionally unimportant quantities of ascorbic acid are found in commercially manufactured evaporated milk after two months of storage. It is commercially feasible and economically sound to add 50 to 100 mg. per liter of ascorbic acid (reconstituted basis) to fortified Vitamin D evaporated milk. Losses of ascorbic acid in evaporated milk may be minimized by sealing the cans in the absence of air.

Little or no dehydroascorbic acid is found in evaporated milk as it is relatively unstable as compared to the reduced form in the canned product.

Natural aging, accelerated by increases in temperature, produces increased amounts of reducing substances in evaporated milk as well as more pronounced flavor, darker color, greater salt deposit and lower pH. The rate of loss of ascorbic acid in evaporated milk is increased by raising the storage temperature but by no constant amount per each 10° C. raise in temperature. There is no significant loss of ascorbic acid in concentrated milk if fortified and held for 24 hours before filling and sterilizing. The sterilizing process inactivates copper in evaporated milk so that it does not act as an oxidative catalyst and has no effect upon rate of loss and ascorbic acid or generation of reducing substances.

The degree of etching or spangling of the inner, tinned surface of the cans of evaporated milk during storage is inversely related to the amount of copper present in the milk. During storage the copper content decreases, due probably to electro-chemical changes.

Destabilization of the milk proteins occurs with high level fortification of evaporated milk with ascorbic acid, but it can be readily corrected by the use of either of the stabilizing salts, sodium citrate, disodium phosphate or sodium carbonate. The use of sodium l-ascorbate for fortification in place of ascorbic acid also prevents destabilization of the milk proteins during sterilization. H.P.

HERD MANAGEMENT

390. A Farm Management Study of 91 Dairy Farms in the Vicinity of Milton, Vermont, 1942. SHELDON W. WILLIAMS. Vermont Agr. Expt. Sta. Bul. 519. 19 pages. January, 1945.

This study of the business operations of 91 farms in the vicinity of Milton during 1942 gives information about incomes, and factors affecting incomes, on dairy farms at a time when the price of milk had recovered from the low levels of the thirties. As natural conditions of the region are suited to dairying and a good fluid milk market is available, the farms are highly specialized in milk production, with slightly more than four-fifths of their receipts coming from that source. In 1942, they were being operated under price conditions similar to those of 1925–29, with the price of milk higher in relation to costs of production than it had been in a dozen years. Despite the improved situation, labor incomes averaged only $322 per farm. They were unusually variable, with a range of more than $8,000 from lowest to highest. A number of factors were responsible for the farm-to-farm variation in returns. Of first rank among them was production per cow. The advantage of having high-producing cows was especially pronounced, having been increased by the rise in the price of milk. Labor efficiency was also of much importance. Farm wages were high in 1942, and the need for getting a large amount of productive work done per man was even greater than usual. Of less consequence, but also important, was the advantage of making effective use of capital. The greater the number of these respects in which the business was well managed, the higher the income; the greater the number in which it was poorly managed, the lower the income. Labor incomes averaged nearly $2,500 higher in the group of farms which were better than average in all three respects than in the group below average in all of them. Incomes were better on large than on small farms. A major reason for this was that efficiency in the use of labor and capital rose considerably with an increase in the size of the business. Large size was not advantageous on poorly managed farms. However, with good management, there was much to be gained by having a large volume of business, and the better the management the greater the advantage in large-scale operation.

A set of business standards for Vermont dairy farms is suggested. 18 tables, 3 figures. J.G.A.

391. Use of Labor on Maine Farms with Dairy Cows. GEORGE F. DOW. Maine Agr. Expt. Sta. Bul. 486. 1945.

This study is based upon a survey made during the summer of 1943 including 500 Maine farmers keeping dairy cows, and other information included was obtained from a total of 989 dairy farmers included in earlier surveys extending from 1928 to 1937.

The average amount of labor used per farm was about 22 months per farm annually. During 1942–43, the farm operator accounted for 49 per cent of the labor, his family an additional 31 per cent, hired year and month help 15 per cent, and hired day help 5 per cent. The number of unpaid family workers who furnished some farm labor averaged 1.26 persons per farm.

Despite loss of young men from farm work, milk production was maintained chiefly because the average herd was not over 11 cows. The working efficiency of farm labor decreased on 0.65 per cent from 88.67 per cent 1942–43.

The efficiency in the use of labor is relatively low on many dairy farms because of the small size of farm business, low rates of production, and a lack of labor-saving equipment. The dairymen with the most efficient use of labor averaged 1.7 men per farm, kept 14.4 cows, and had a labor income of + $394 per farm. The least efficient, with 1.8 men per farm, kept only 6.6 cows and had a labor income of – $406 per farm.

The farmers with the highest crop yields and largest milk production per cow produced 70 per cent more food per man, and had a labor income of + $330 per farm, as compared with – $305 for the least productive farmers.

Milking machines, buck rakes, and watering cups were some of the types of equipment that resulted in more efficient use of labor.

The farmers who supplemented dairying with some other farm business used labor more efficiently and had a larger labor income than those who specialized in dairying. L.M.D.

ICE CREAM

392. What's Ahead? Will You Face Super Market Competition? ANONYMOUS. Ice Cream Field, *46,* No. 2: 28, 29. 1945.

Data are presented to show that at least one "super market" is making a profit selling ice cream at 15 cents a pint. It is stated that retailers in the ice cream industry are carefully watching the developments in the distribution of package ice cream through "super markets," and some are considering the merchandising of package ice cream through self-service cabinets. W.C.C.

MILK

393. Fluid Milk and Fluid Cream Sales, Allegheny County, 1936–1944. C. W. PIERCE. Penn. Agr. Expt. Sta. Bul. 471. 21 pages. Feb., 1944.

Increased per capita consumption accounts for approximately 40 per cent gain in fluid milk sales in Allegheny County (Pittsburgh) from middle of 1939 to the middle of 1944. From May, 1939, to May, 1944, the payroll

index increased 165 points. The value of daily milk sales increased $32,000. Each 10-point change in the index was accompanied by a change in the value of milk sold of approximately $2,000. Economic prosperity for the fluid milk industry resulted from high levels of milk consumption accompanying large wartime consumer incomes. The farmer as well as the processing plant has prospered with the gain in milk sales. The post-war position of the milk industry will be largely determined by what happens to consumer incomes. The industry should give consideration to (1) an expanded program of nutrition education, (2) possibilities of reducing costs in the production and distribution of milk, (3) prompt price adjustments in order to promote stability of supplies and sales, and (4) consumer education with respect to the effects of removing the present consumer subsidies. H.P.

394. Churns vs. Tankers for Bulk Haulage. J. INGLIS. The Milk. Industry, *26*, No. 2: 37. August, 1945.

The transportation of milk falls into two categories (1) transport in small containers, known variously as churns, kits, cans, and tankards, and (2) transport in large containers.

The problem of design of trucks, cans and tanks merit closer study than they commonly receive. Milk transport is a seven day a week job. Maintenance of the vehicles used must be carefully planned and supervised. It will pay the industry well to avoid carrying unnecessary dead weight. The possibility of using light metal alloys at all stages merits study. The larger the single bulk that can be used at all stages, the greater the economies in transporting and handling milk in the dairies. H.P.

MISCELLANEOUS

395. Freezing Rates of Foods. J. E. NICHOLAS. Penn. Agr. Expt. Sta. Bul. 471. 20 pages. Feb., 1945.

Data were obtained on vegetables, poultry and meats on rates of freezing in farm- and domestic-type food storage cabinets and in locker plants employing single-plate and air-blast methods of freezing. Products placed to freeze went through three different stages: precooling, freezing, and subcooling. The time interval required for precooling varied from 1¼ to 2 hours. The freezing interval varied from 2 to 12 or more hours, depending on size of the package and number of layers of wrapping material. Products which were frozen attained the temperature of the plate or of the air in the freezer within 8 to 24 hours depending on the size of the package and the number of wrapping material. With a plate temperature variation of approximately 6° F., the temperature of a frozen product in the freezing compartment varied less than 1° F. H.P.

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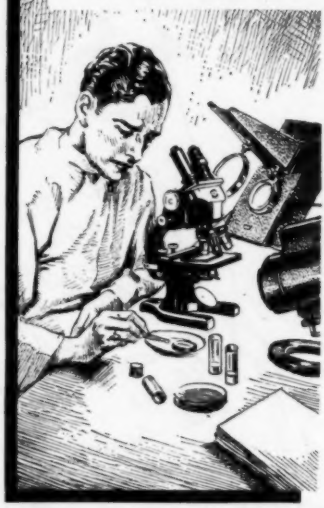

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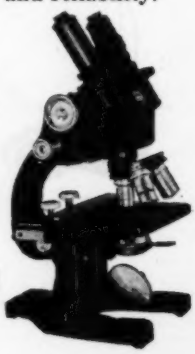

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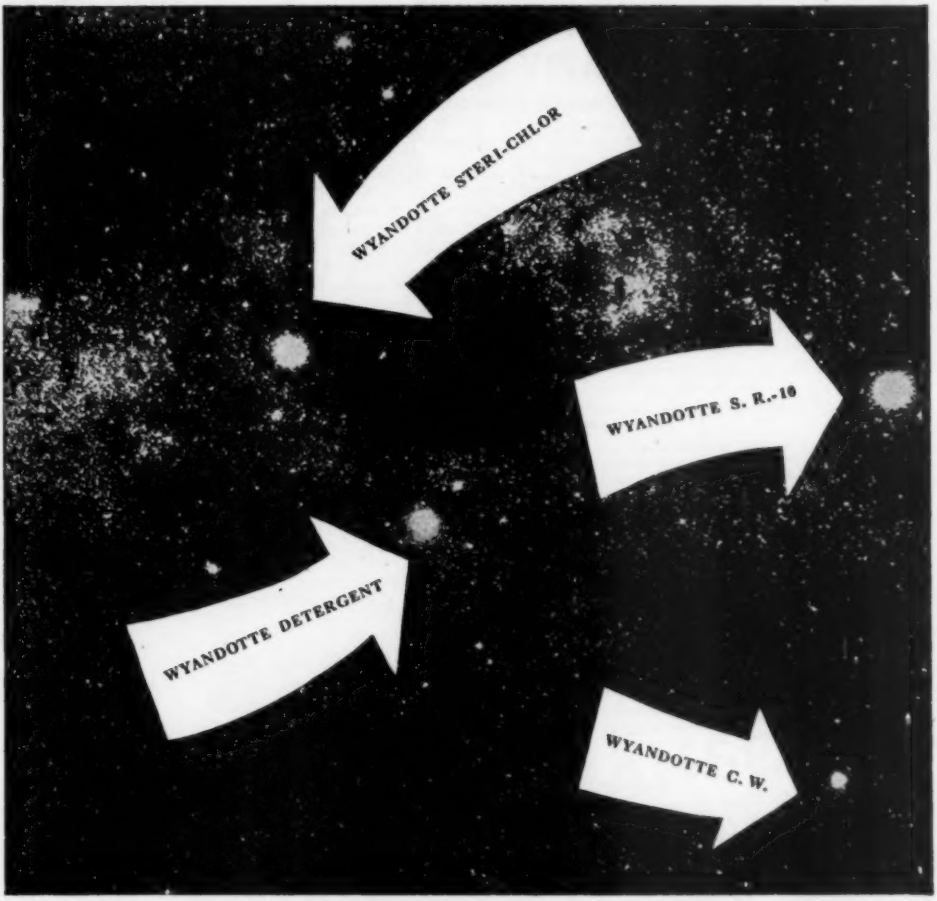

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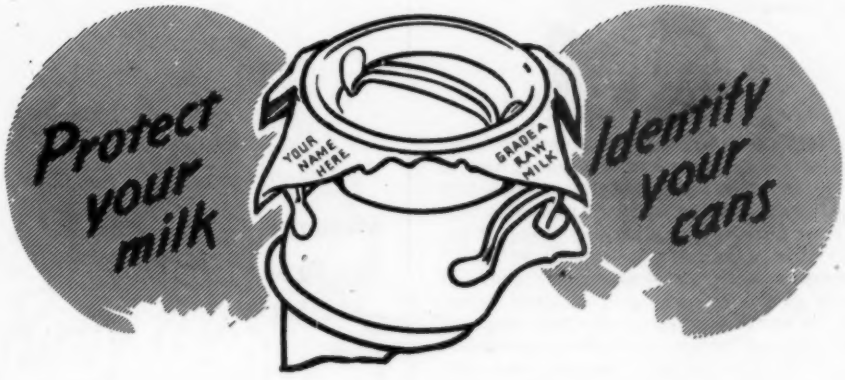

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SAVOR	.....	**b** ☐
AVENGE	.....	**c** ☐
STIPEND	.....	**d** ☐
LINK	.....	**e** ☐
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b — 9
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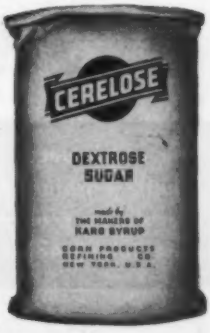

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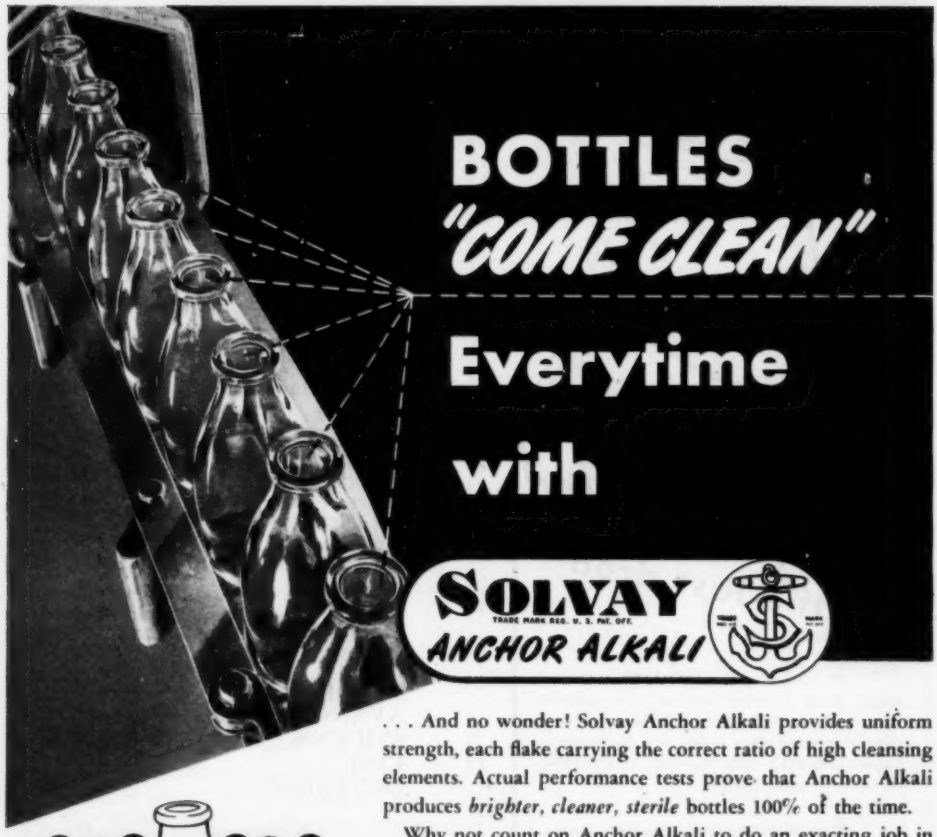

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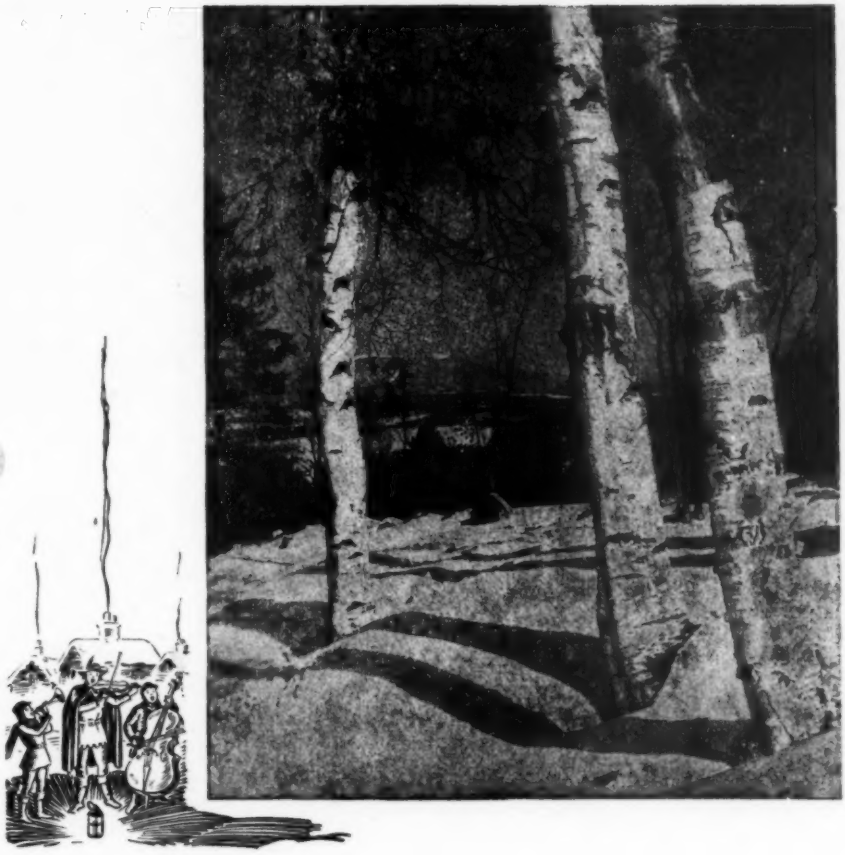

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